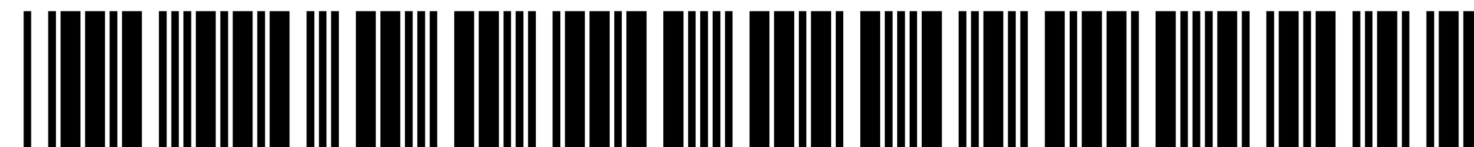
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Metz et al.

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(45) **Date of Patent: *Oct. 20, 2009**

(54) **PUFA POLYKETIDE SYNTHASE SYSTEMS AND USES THEREOF**

(75) Inventors: **James G. Metz**, Longmont, CO (US); **James H. Flatt**, Colorado Springs, CO (US); **Jerry M. Kuner**, Longmont, CO (US); **William R. Barclay**, Boulder, CO (US)

(73) Assignee: **Martek Biosciences Corporation**, Columbia, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(60) Provisional application No. 60/284,066, filed on Apr. 16, 2001, provisional application No. 60/298,796, filed on Jun. 15, 2001, provisional application No. 60/323,269, filed on Sep. 18, 2001.

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C12N 15/82	(2006.01)
C12P 7/64	(2006.01)

(52) **U.S. Cl.** **536/23.2**; 435/234; 435/183; 435/189; 435/252.3; 435/320.1; 435/410

(58) **Field of Classification Search** .... None
See application file for complete search history.

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Primary Examiner—Anand U. Desai
Assistant Examiner—William W Moore
(74) *Attorney, Agent, or Firm*—Sheridan Ross P.C.

(57) **ABSTRACT**

The invention generally relates to polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) systems isolated from or derived from non-bacterial organisms, to homologues thereof, to isolated nucleic acid molecules and recombinant nucleic acid molecules encoding biologically active domains of such a PUFA PKS system, to genetically modified organisms comprising PUFA PKS systems, to methods of making and using such systems for the production of bioactive molecules of interest, and to novel methods for identifying new bacterial and non-bacterial microorganisms having such a PUFA PKS system.

23 Claims, 3 Drawing Sheets

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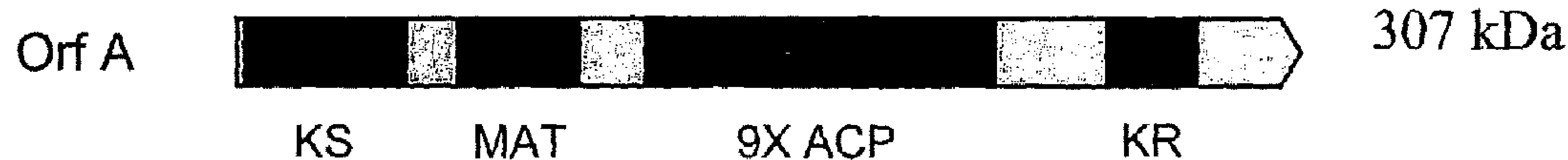

Orf B

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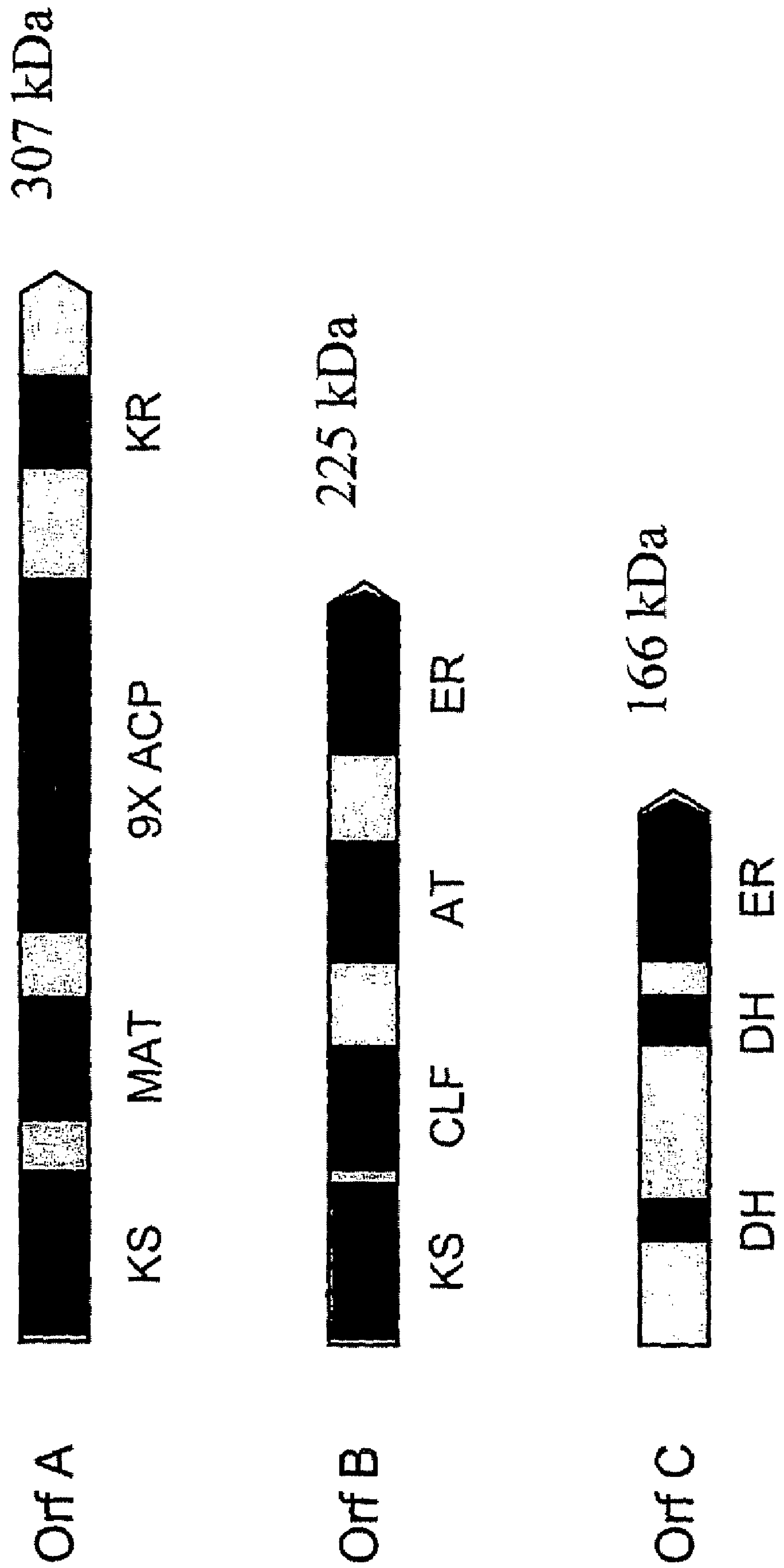
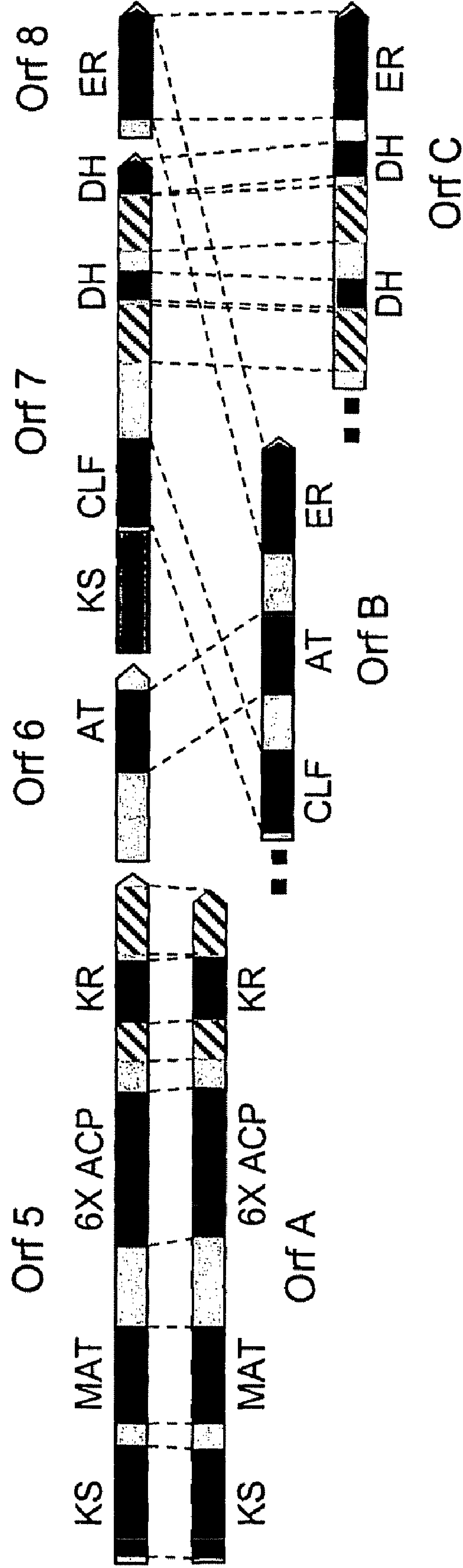


FIG. 1

FIG. 2

Comparison of PKS Orfs/domains:

Schizochytrium vs *Shewanella*

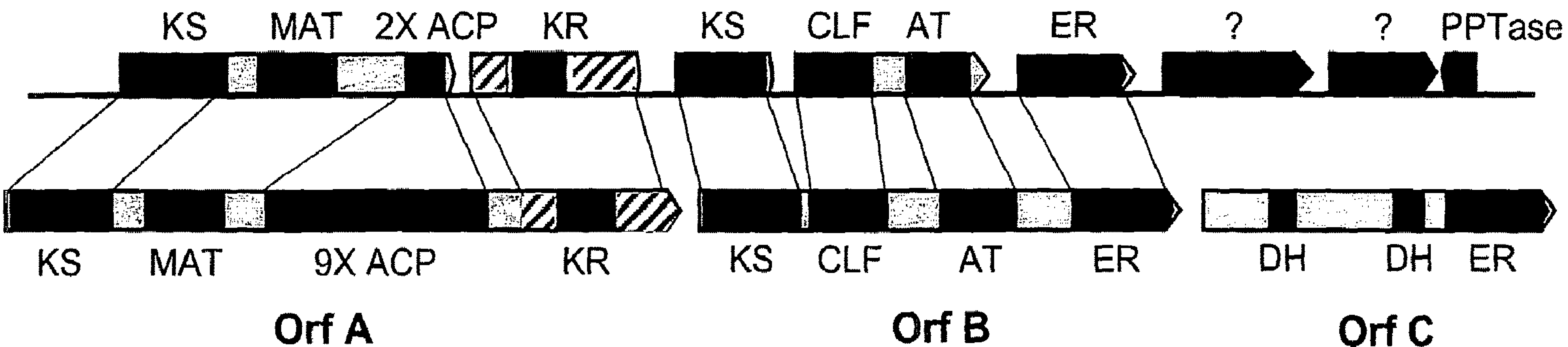


Amino acid sequence homology

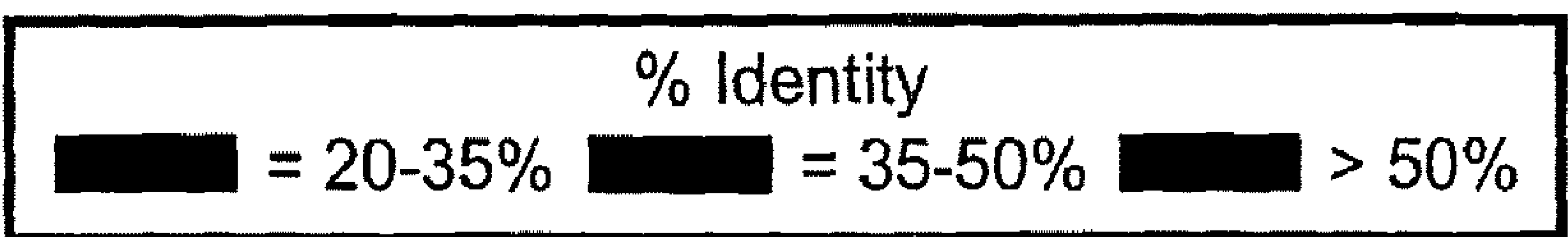
FIG. 3

Nostoc* (7120) vs *Schizochytrium

***Nostoc* ORFs in the Hgl/Het cluster**
(linear order on the chromosome)



***Schizochytrium* PUFA PKS ORFs**
(separate Orfs)



PUFA POLYKETIDE SYNTHASE SYSTEMS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 10/124,800, filed Apr. 16, 2002, now U.S. Pat. No. 7,247,461, entitled "PUFA Polyketide Synthase Systems and Uses Thereof," which claims the benefit of priority under 35 U.S.C. § 119(e) to: U.S. Provisional Application Ser. No. 60/284,066, filed Apr. 16, 2001, entitled "A Polyketide Synthase System and Uses Thereof"; U.S. Provisional Application Ser. No. 60/298,796, filed Jun. 15, 2001, entitled "A Polyketide Synthase System and Uses Thereof"; and U.S. Provisional Application Ser. No. 60/323,269, filed Sep. 18, 2001, entitled "*Thraustochytrium* PUFA PKS System and Uses Thereof". U.S. application Ser. No. 10/124,800, is also a continuation-in-part of U.S. application Ser. No. 09/231,899, now U.S. Pat. No. 6,566,583, filed Jan. 14, 1999, entitled "Schizochytrium PKS Genes". Each of the above-identified patent applications is incorporated herein by reference in its entirety.

This application does not claim the benefit of priority from U.S. application Ser. No. 09/090,793, filed Jun. 4, 1998, now U.S. Pat. No. 6,140,486, although U.S. application Ser. No. 09/090,793 is incorporated herein by reference in its entirety.

REFERENCE TO SEOUENCE LISTING

This application contains a Sequence Listing submitted as an electronic text file named "2997-29_corrected_ST25. txt", having a size in bytes of 280 kb, and created on 4 Mar. 2007. The information contained in this electronic file is hereby incorporated by reference in its entirety pursuant to 37 CFR §1.52(e)(5).

FIELD OF THE INVENTION

This invention relates to polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) systems from microorganisms, including eukaryotic organisms, such as Thraustochytrid microorganisms. More particularly, this invention relates to nucleic acids encoding non-bacterial PUFA PKS systems, to non-bacterial PUFA PKS systems, to genetically modified organisms comprising non-bacterial PUFA PKS systems, and to methods of making and using the non-bacterial PUFA PKS systems disclosed herein. This invention also relates to a method to identify bacterial and non-bacterial microorganisms comprising PUFA PKS systems.

BACKGROUND OF THE INVENTION

Polyketide synthase (PKS) systems are generally known in the art as enzyme complexes derived from fatty acid synthase (FAS) systems, but which are often highly modified to produce specialized products that typically show little resemblance to fatty acids. Researchers have attempted to exploit polyketide synthase (PKS) systems that have been described in the literature as falling into one of three basic types, typically referred to as: Type II, Type I and modular. The Type II system is characterized by separable proteins, each of which carries out a distinct enzymatic reaction. The enzymes work in concert to produce the end product and each individual enzyme of the system typically participates several times in the production of the end product. This type of system operates in a manner analogous to the fatty acid synthase (FAS) systems found in plants and bacteria. Type I PKS systems are similar to the Type II system in that the enzymes are used in an iterative fashion to produce the end product. The Type I differs from Type II in that enzymatic activities, instead of being associated with separable proteins, occur as domains of larger proteins. This system is analogous to the Type I FAS systems found in animals and fungi.

In contrast to the Type I and II systems, in modular PKS systems, each enzyme domain is used only once in the production of the end product. The domains are found in very large proteins and the product of each reaction is passed on to another domain in the PKS protein. Additionally, in all of the PKS systems described above, if a carbon-carbon double bond is incorporated into the end product, it is always in the trans configuration.

In the Type I and Type II PKS systems described above, the same set of reactions is carried out in each cycle until the end product is obtained. There is no allowance for the introduction of unique reactions during the biosynthetic procedure. The modular PKS systems require huge proteins that do not utilize the economy of iterative reactions (i.e., a distinct domain is required for each reaction). Additionally, as stated above, carbon-carbon double bonds are introduced in the trans configuration in all of the previously described PKS systems.

Polyunsaturated fatty acids (PUFAs) are critical components of membrane lipids in most eukaryotes (Lauritzen et al., *Prog. Lipid Res.* 40 1 (2001); McConn et al., *Plant J.* 15, 521 (1998)) and are precursors of certain hormones and signaling molecules (Heller et al., *Drugs* 55, 487 (1998); Creelman et al., *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 355 (1997)). Known pathways of PUFA synthesis involve the processing of saturated 16:0 or 18:0 fatty acids (the abbreviation X:Y indicates an acyl group containing X carbon atoms and Y cis double bonds; double-bond positions of PUFAs are indicated relative to the methyl carbon of the fatty acid chain (ω3 or ω6) with systematic methylene interruption of the double bonds) derived from fatty acid synthase (FAS) by elongation and aerobic desaturation reactions (Sprecher, *Curr. Opin. Clin. Nutr. Metab. Care* 2, 135 (1999); Parker-Barnes et al., *Proc. Natl. Acad. Sci. USA* 97, 8284 (2000); Shanklin et al., *Annu. Rev. Plant Physiol. Plant Nol. Biol.* 49, 611 (1998)). Starting from acetyl-CoA, the synthesis of DHA requires approximately 30 distinct enzyme activities and nearly 70 reactions including the four repetitive steps of the fatty acid synthesis cycle. Polyketide synthases (PKSs) carry out some of the same reactions as FAS (Hopwood et al., *Annu. Rev. Genet.* 24, 37 (1990); Bentley et al., *Annu. Rev. Microbiol.* 53, 411 (1999)) and use the same small protein (or domain), acyl carrier protein (ACP), as a covalent attachment site for the growing carbon chain. However, in these enzyme systems, the complete cycle of reduction, dehydration and reduction seen in FAS is often abbreviated so that a highly derivatized carbon chain is produced, typically containing many keto- and hydroxy-groups as well as carbon-carbon double bonds in the trans configuration. The linear products of PKSs are often cyclized to form complex biochemicals that include antibiotics and many other secondary products (Hopwood et al., (1990) supra; Bentley et al., (1999), supra; Keating et al., *Curr. Opin. Chem. Biol.* 3, 598 (1999)).

Very long chain PUFAs such as docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3) have been reported from several species of marine bacteria, including *Shewanella* sp (Nichols et al., *Curr. Op. Biotechnol.* 10, 240 (1999); Yazawa, *Lipids* 31, S (1996); DeLong et al., *Appl. Environ. Microbiol.* 51, 730 (1986)). Analysis of a genomic fragment (cloned as plasmid pEPA) from *Shewanella* sp. strain SCRC2738 led to the identification of

five open reading frames (Orfs), totaling 20 Kb, that are necessary and sufficient for EPA production in *E. coli* (Yazawa, (1996), supra). Several of the predicted protein domains were homologues of FAS enzymes, while other regions showed no homology to proteins of known function. On the basis of these observations and biochemical studies, it was suggested that PUFA synthesis in *Shewanella* involved the elongation of 16- or 18-carbon fatty acids produced by FAS and the insertion of double bonds by undefined aerobic desaturases (Watanabe et al., *J. Biochem.* 122, 467 (1997)). The recognition that this hypothesis was incorrect began with a reexamination of the protein sequences encoded by the five *Shewanella* Orfs. At least 11 regions within the five Orfs were identifiable as putative enzyme domains (See Metz et al., *Science* 293:290-293 (2001)). When compared with sequences in the gene databases, seven of these were more strongly related to PKS proteins than to FAS proteins. Included in this group were domains putatively encoding malonyl-CoA:ACP acyltransferase (MAT), 3-ketoacyl-ACP synthase (KS), 3-ketoacyl-ACP reductase (KR), acyltransferase (AT), phosphopantetheine transferase, chain length (or chain initiation) factor (CLF) and a highly unusual cluster of six ACP domains (i.e., the presence of more than two clustered ACP domains has not previously been reported in PKS or FAS sequences). However, three regions were more highly homologous to bacterial FAS proteins. One of these was similar to the newly-described Triclosan-resistant enoyl reductase (ER) from *Streptococcus pneumoniae* (Heath et al., *Nature* 406, 145 (2000)); comparison of ORF8 peptide with the *S. pneumoniae* enoyl reductase using the LALIGN program (matrix, BLOSUM50; gap opening penalty, −10; elongation penalty −1) indicated 49% similarity over a 386aa overlap). Two regions were homologues of the *E. coli* FAS protein encoded by fabA, which catalyzes the synthesis of trans-2-decenoyl-ACP and the reversible isomerization of this product to cis-3-decenoyl-ACP (Heath et al., *J. Biol. Chem.,* 271, 27795 (1996)). On this basis, it seemed likely that at least some of the double bonds in EPA from *Shewanella* are introduced by a dehydrase-isomerase mechanism catalyzed by the FabA-like domains in Orf7.

Anaerobically-grown *E. coli* cells harboring the pEPA plasmid accumulated EPA to the same levels as aerobic cultures (Metz et al., 2001, supra), indicating that an oxygen-dependent desaturase is not involved in EPA synthesis. When pEPA was introduced into a fabB$^-$ mutant of *E. coli*, which is unable to synthesize monounsaturated fatty acids and requires unsaturated fatty acids for growth, the resulting cells lost their fatty acid auxotrophy.

They also accumulated much higher levels of EPA than other pEPA-containing strains, suggesting that EPA competes with endogenously produced monounsaturated fatty acids for transfer to glycerolipids. When pEPA-containing *E. coli* cells were grown in the presence of [^{13}C]-acetate, the data from ^{13}C-NMR analysis of purified EPA from the cells confirmed the identity of EPA and provided evidence that this fatty acid was synthesized from acetyl-CoA and malonyl-CoA (See Metz et al., 2001, supra). A cell-free homogenate from pEPA-containing fabB$^-$ cells synthesized both EPA and saturated fatty acids from [^{14}C]-malonyl-CoA. When the homogenate was separated into a 200,000×g high-speed pellet and a membrane-free supernatant fraction, saturated fatty acid synthesis was confined to the supernatant, consistent with the soluble nature of the Type II FAS enzymes (Magnuson et al., *Microbiol. Rev.* 57, 522 (1993)). Synthesis of EPA was found only in the high-speed pellet fraction, indicating that EPA synthesis can occur without reliance on enzymes of the *E. coli* FAS or on soluble intermediates (such as 16:0-ACP) from the cytoplasmic fraction. Since the proteins encoded by the *Shewanella* EPA genes are not particularly hydrophobic, restriction of EPA synthesis activity to this fraction may reflect a requirement for a membrane-associated acyl acceptor molecule. Additionally, in contrast to the *E. coli* FAS, EPA synthesis is specifically NADPH-dependent and does not require NADH. All these results are consistent with the pEPA genes encoding a multifunctional PKS that acts independently of FAS, elongase, and desaturase activities to synthesize EPA directly. It is likely that the PKS pathway for PUFA synthesis that has been identified in *Shewanella* is widespread in marine bacteria. Genes with high homology to the *Shewanella* gene cluster have been identified in *Photobacterium* profundum (Allen et al., *Appli. Environ. Microbiol.* 65:1710 (1999)) and in *Moritella marina* (*Vibrio marinus*) (Tanaka et al., *Biotechnol. Lett.* 21:939 (1999)).

The biochemical and molecular-genetic analyses performed with *Shewanella* provide compelling evidence for polyketide synthases that are capable of synthesizing PUFAs from malonyl-CoA. A complete scheme for synthesis of EPA by the *Shewanella* PKS has been proposed. The identification of protein domains homologous to the *E. coli* FabA protein, and the observation that bacterial EPA synthesis occurs anaerobically, provide evidence for one mechanism wherein the insertion of cis double bonds occurs through the action of a bifunctional dehydratase/2-trans, 3-cis isomerase (DH/2, 3I). In *E. coli*, condensation of the 3-cis acyl intermediate with malonyl-ACP requires a particular ketoacyl-ACP synthase and this may provide a rationale for the presence of two KS in the *Shewanella* gene cluster (in Orf 5 and Orf 7). However, the PKS cycle extends the chain in two-carbon increments while the double bonds in the EPA product occur at every third carbon. This disjunction can be solved if the double bonds at C-14 and C-8 of EPA are generated by 2-trans, 2-cis isomerization (DH/2,2I) followed by incorporation of the cis double bond into the elongating fatty acid chain. The enzymatic conversion of a trans double bond to the cis configuration without bond migration is known to occur, for example, in the synthesis of 11-cis-retinal in the retinoid cycle (Jang et al., *J. Biol. Chem.* 275, 28128 (2000)). Although such an enzyme function has not yet been identified in the *Shewanella* PKS, it may reside in one of the unassigned protein domains.

The PKS pathways for PUFA synthesis in *Shewanella* and another marine bacteria, *Vibrio marinus*, are described in detail in U.S. Pat. No. 6,140,486 (issued from U.S. application Ser. No. 09/090,793, filed Jun. 4, 1998, entitled "Production of Polyunsaturated Fatty Acids by Expression of Polyketide-like Synthesis Genes in Plants", which is incorporated herein by reference in its entirety).

Polyunsaturated fatty acids (PUFAs) are considered to be useful for nutritional, pharmaceutical, industrial, and other purposes. An expansive supply of PUFAs from natural sources and from chemical synthesis are not sufficient for commercial needs. Because a number of separate desaturase and elongase enzymes are required for fatty acid synthesis from linoleic acid (LA, 18:2 Δ 9, 12), common in most plant species, to the more saturated and longer chain PUFAs, engineering plant host cells for the expression of PUFAs such as EPA and DHA may require expression of five or six separate enzyme activities to achieve expression, at least for EPA and DHA. Additionally, for production of useable quantities of such PUFAs, additional engineering efforts may be required, for instance the down regulation of enzymes competing for substrate, engineering of higher enzyme activities such as by mutagenesis or targeting of enzymes to plastid organelles. Therefore it is of interest to obtain genetic material involved

in PUFA biosynthesis from species that naturally produce these fatty acids and to express the isolated material alone or in combination in a heterologous system which can be manipulated to allow production of commercial quantities of PUFAs.

The discovery of a PUFA PKS system in marine bacteria such as *Shewanella* and *Vibrio marinus* (see U.S. Pat. No. 6,140,486, ibid.) provides a resource for new methods of commercial PUFA production. However, these marine bacteria have limitations which will ultimately restrict their usefulness on a commercial level. First, although U.S. Pat. No. 6,140,486 discloses that the marine bacteria PUFA PKS systems can be used to genetically modify plants, the marine bacteria naturally live and grow in cold marine environments and the enzyme systems of these bacteria do not function well above 30° C. In contrast, many crop plants, which are attractive targets for genetic manipulation using the PUFA PKS system, have normal growth conditions at temperatures above 30° C. and ranging to higher than 40° C. Therefore, the marine bacteria PUFA PKS system is not predicted to be readily adaptable to plant expression under normal growth conditions. Moreover, the marine bacteria PUFA PKS genes, being from a bacterial source, may not be compatible with the genomes of eukaryotic host cells, or at least may require significant adaptation to work in eukaryotic hosts. Additionally, the known marine bacteria PUFA PKS systems do not directly produce triglycerides, whereas direct production of triglycerides would be desirable because triglycerides are a lipid storage product in microorganisms and as a result can be accumulated at very high levels (e.g. up to 80-85% of cell weight) in microbial/plant cells (as opposed to a “structural” lipid product (e.g. phospholipids) which can generally only accumulate at low levels (e.g. less than 10-15% of cell weight at maximum)).

Therefore, there is a need in the art for other PUFA PKS systems having greater flexibility for commercial use.

SUMMARY OF THE INVENTION

One embodiment of the present invention relates to an isolated nucleic acid molecule comprising a nucleic acid sequence chosen from: (a) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and biologically active fragments thereof; (b) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (c) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of the amino acid sequence of (a), wherein the amino acid sequence has a biological activity of at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system; (d) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to the amino acid sequence of (b), wherein the amino acid sequence has a biological activity of at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system; and (e) a nucleic acid sequence that is fully complementary to the nucleic acid sequence of (a), (b), (c), or (d). In alternate aspects, the nucleic acid sequence encodes an amino acid sequence that is at least about 70% identical, or at least about 80% identical, or at least about 90% identical, or is identical to: (1) at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; and/or (2) a nucleic acid sequence encoding an amino acid sequence that is at least about 70% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32. In a preferred embodiment, the nucleic acid sequence encodes an amino acid sequence chosen from: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:288, SEQ ID NO:30, SEQ ID NO:32 and/or biologically active fragments thereof. In one aspect, the nucleic acid sequence is chosen from: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 12, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, and SEQ ID NO:31.

Another embodiment of the present invention relates to a recombinant nucleic acid molecule comprising the nucleic acid molecule as described above, operatively linked to at least one transcription control sequence. In another embodiment, the present invention relates to a recombinant cell transfected with the recombinant nucleic acid molecule described directly above.

Yet another embodiment of the present invention relates to a genetically modified microorganism, wherein the microorganism expresses a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The at least one domain of the PUFA PKS system is encoded by a nucleic acid sequence chosen from: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS system from a microorganism identified by the screening method of the present invention; (c) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6, and biologically active fragments thereof; (d) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (e) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and, (f) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. In this embodiment, the microorganism is genetically modified to affect the activity of the PKS system. The screening method of the present invention referenced in (b) above comprises: (i) selecting a microorganism that produces at least one PUFA; and, (ii) identifying a microorganism from (i) that has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation in the fermentation medium, as compared to production

of PUFAs by the microorganism under dissolved oxygen conditions of greater than 5% of saturation, and more preferably 10% of saturation, and more preferably greater than 15% of saturation, and more preferably greater than 20% of saturation in the fermentation medium.

In one aspect, the microorganism endogenously expresses a PKS system comprising the at least one domain of the PUFA PKS system, and wherein the genetic modification is in a nucleic acid sequence encoding the at least one domain of the PUFA PKS system. For example, the genetic modification can be in a nucleic acid sequence that encodes a domain having a biological activity of at least one of the following proteins: malonyl-CoA:ACP acyltransferase (MAT), β-keto acyl-ACP synthase (KS), ketoreductase (KR), acyltransferase (AT), FabA-like β-hydroxy acyl-ACP dehydrase (DH), phosphopantetheine transferase, chain length factor (CLF), acyl carrier protein (ACP), enoyl ACP-reductase (ER), an enzyme that catalyzes the synthesis of trans-2-decenoyl-ACP, an enzyme that catalyzes the reversible isomerization of trans-2-decenoyl-ACP to cis-3-decenoyl-ACP, and an enzyme that catalyzes the elongation of cis-3-decenoyl-ACP to cis-vaccenic acid. In one aspect, the genetic modification is in a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of: (a) an amino acid sequence that is at least about 70% identical, and preferably at least about 80% identical, and more preferably at least about 90% identical and more preferably identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and, (b) an amino acid sequence that is at least about 70% identical, and preferably at least about 80% identical, and more preferably at least about 90% identical and more preferably identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system.

In one aspect, the genetically modified microorganism is a Thraustochytrid, which can include, but is not limited to, a Thraustochytrid from a genus chosen from *Schizochytrium* and *Thraustochytrium*. In another aspect, the microorganism has been further genetically modified to recombinantly express at least one nucleic acid molecule encoding at least one biologically active domain from a bacterial PUFA PKS system, from a Type I PKS system, from a Type II PKS system, and/or from a modular PKS system.

In another aspect of this embodiment, the microorganism endogenously expresses a PUFA PKS system comprising the at least one biologically active domain of a PUFA PKS system, and wherein the genetic modification comprises expression of a recombinant nucleic acid molecule selected from the group consisting of a recombinant nucleic acid molecule encoding at least one biologically active domain from a second PKS system and a recombinant nucleic acid molecule encoding a protein that affects the activity of the PUFA PKS system. Preferably, the recombinant nucleic acid molecule comprises any one of the nucleic acid sequences described above.

In one aspect of this embodiment, the recombinant nucleic acid molecule encodes a phosphopantetheine transferase. In another aspect, the recombinant nucleic acid molecule comprises a nucleic acid sequence encoding at least one biologically active domain from a bacterial PUFA PKS system, from a type I PKS system, from a type II PKS system, and/or from a modular PKS system.

In another aspect of this embodiment, the microorganism is genetically modified by transfection with a recombinant nucleic acid molecule encoding the at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. Such a recombinant nucleic acid molecule can include any recombinant nucleic acid molecule comprising any of the nucleic acid sequences described above. In one aspect, the microorganism has been further genetically modified to recombinantly express at least one nucleic acid molecule encoding at least one biologically active domain from a bacterial PUFA PKS system, from a Type I PKS system, from a Type II PKS system, or from a modular PKS system.

Yet another embodiment of the present invention relates to a genetically modified plant, wherein the plant has been genetically modified to recombinantly express a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The domain can be encoded by any of the nucleic acid sequences described above. In one aspect, the plant has been further genetically modified to recombinantly express at least one nucleic acid molecule encoding at least one biologically active domain from a bacterial PUFA PKS system, from a Type I PKS system, from a Type II PKS system, and/from a modular PKS system.

Another embodiment of the present invention relates to a method to identify a microorganism that has a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The method includes the steps of: (a) selecting a microorganism that produces at least one PUFA; and, (b) identifying a microorganism from (a) that has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation in the fermentation medium, as compared to production of PUFAs by the microorganism under dissolved oxygen conditions of greater than 5% of saturation, more preferably 10% of saturation, more preferably greater than 15% of saturation and more preferably greater than 20% of saturation in the fermentation medium. A microorganism that produces at least one PUFA and has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation is identified as a candidate for containing a PUFA PKS system.

In one aspect of this embodiment, step (b) comprises identifying a microorganism from (a) that has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 2% of saturation, and more preferably under dissolved oxygen conditions of less than about 1% of saturation, and even more preferably under dissolved conditions of about 0% of saturation.

In another aspect of this embodiment, the microorganism selected in (a) has an ability to consume bacteria by phagocytosis. In another aspect, the microorganism selected in (a) has a simple fatty acid profile. In another aspect, the microorganism selected in (a) is a non-bacterial microorganism. In another aspect, the microorganism selected in (a) is a eukaryote. In another aspect, the microorganism selected in (a) is a member of the order Thraustochytriales. In another aspect, the microorganism selected in (a) has an ability to produce PUFAs at a temperature greater than about 15° C., and preferably greater than about 20° C., and more preferably greater than about 25° C., and even more preferably greater than about 30° C. In another aspect, the microorganism selected in (a) has an ability to produce bioactive compounds (e.g., lipids) of interest at greater than 5% of the dry weight of the organism, and more preferably greater than 10% of the dry

weight of the organism. In yet another aspect, the microorganism selected in (a) contains greater than 30% of its total fatty acids as C14:0, C16:0 and C16:1 while also producing at least one long chain fatty acid with three or more unsaturated bonds, and preferably, the microorganism selected in (a) contains greater than 40% of its total fatty acids as C14:0, C16:0 and C16:1 while also producing at least one long chain fatty acid with three or more unsaturated bonds. In another aspect, the microorganism selected in (a) contains greater than 30% of its total fatty acids as C14:0, C16:0 and C16:1 while also producing at least one long chain fatty acid with four or more unsaturated bonds, and more preferably while also producing at least one long chain fatty acid with five or more unsaturated bonds.

In another aspect of this embodiment, the method further comprises step (c) of detecting whether the organism comprises a PUFA PKS system. In this aspect, the step of detecting can include detecting a nucleic acid sequence in the microorganism that hybridizes under stringent conditions with a nucleic acid sequence encoding an amino acid sequence from a Thraustochytrid PUFA PKS system. Alternatively, the step of detecting can include detecting a nucleic acid sequence in the organism that is amplified by oligonucleotide primers from a nucleic acid sequence from a Thaustochytrid PUFA PKS system.

Another embodiment of the present invention relates to a microorganism identified by the screening method described above, wherein the microorganism is genetically modified to regulate the production of molecules by the PUFA PKS system.

Yet another embodiment of the present invention relates to a method to produce a bioactive molecule that is produced by a polyketide synthase system. The method includes the step of culturing under conditions effective to produce the bioactive molecule a genetically modified organism that expresses a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The domain of the PUFA PKS system is encoded by any of the nucleic acid sequences described above.

In one aspect of this embodiment, the organism endogenously expresses a PKS system comprising the at least one domain of the PUFA PKS system, and the genetic modification is in a nucleic acid sequence encoding the at least one domain of the PUFA PKS system. For example, the genetic modification can change at least one product produced by the endogenous PKS system, as compared to a wild-type organism.

In another aspect of this embodiment, the organism endogenously expresses a PKS system comprising the at least one biologically active domain of the PUFA PKS system, and the genetic modification comprises transfection of the organism with a recombinant nucleic acid molecule selected from the group consisting of: a recombinant nucleic acid molecule encoding at least one biologically active domain from a second PKS system and a recombinant nucleic acid molecule encoding a protein that affects the activity of the PUFA PKS system. For example, the genetic modification can change at least one product produced by the endogenous PKS system, as compared to a wild-type organism.

In yet another aspect of this embodiment, the organism is genetically modified by transfection with a recombinant nucleic acid molecule encoding the at least one domain of the polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. In another aspect, the organism produces a polyunsaturated fatty acid (PUFA) profile that differs from the naturally occurring organism without a genetic modification. In another aspect, the organism endogenously expresses a non-bacterial PUFA PKS system, and wherein the genetic modification comprises substitution of a domain from a different PKS system for a nucleic acid sequence encoding at least one domain of the non-bacterial PUFA PKS system.

In yet another aspect, the organism endogenously expresses a non-bacterial PUFA PKS system that has been modified by transfecting the organism with a recombinant nucleic acid molecule encoding a protein that regulates the chain length of fatty acids produced by the PUFA PKS system. For example, the recombinant nucleic acid molecule encoding a protein that regulates the chain length of fatty acids can replace a nucleic acid sequence encoding a chain length factor in the non-bacterial PUFA PKS system. In another aspect, the protein that regulates the chain length of fatty acids produced by the PUFA PKS system is a chain length factor. In another aspect, the protein that regulates the chain length of fatty acids produced by the PUFA PKS system is a chain length factor that directs the synthesis of C20 units.

In one aspect, the organism expresses a non-bacterial PUFA PKS system comprising a genetic modification in a domain chosen from: a domain encoding FabA-like β-hydroxy acyl-ACP dehydrase (DH) domain and a domain encoding β-ketoacyl-ACP synthase (KS), wherein the modification alters the ratio of long chain fatty acids produced by the PUFA PKS system as compared to in the absence of the modification. In one aspect, the modification comprises substituting a DH domain that does not possess isomerization activity for a FabA-like β-hydroxy acyl-ACP dehydrase (DH) in the non-bacterial PUFA PKS system. In another aspect, the modification is selected from the group consisting of a deletion of all or a part of the domain, a substitution of a homologous domain from a different organism for the domain, and a mutation of the domain.

In another aspect, the organism expresses a PKS system and the genetic modification comprises substituting a FabA-like β-hydroxy acyl-ACP dehydrase (DH) domain from a PUFA PKS system for a DH domain that does not posses isomerization activity.

In another aspect, the organism expresses a non-bacterial PUFA PKS system comprising a modification in an enoyl-ACP reductase (ER) domain, wherein the modification results in the production of a different compound as compared to in the absence of the modification. For example, the modification can be selected from the group consisting of a deletion of all or a part of the ER domain, a substitution of an ER domain from a different organism for the ER domain, and a mutation of the ER domain.

In one aspect, the bioactive molecule produced by the present method can include, but is not limited to, an anti-inflammatory formulation, a chemotherapeutic agent, an active excipient, an osteoporosis drug, an anti-depressant, an anti-convulsant, an anti-*Heliobactor pylori* drug, a drug for treatment of neurodegenerative disease, a drug for treatment of degenerative liver disease, an antibiotic, and a cholesterol lowering formulation. In one aspect, the bioactive molecule is a polyunsaturated fatty acid (PUFA). In another aspect, the bioactive molecule is a molecule including carbon-carbon double bonds in the cis configuration. In another aspect, the bioactive molecule is a molecule including a double bond at every third carbon.

In one aspect of this embodiment, the organism is a microorganism, and in another aspect, the organism is a plant.

Another embodiment of the present invention relates to a method to produce a plant that has a polyunsaturated fatty acid (PUFA) profile that differs from the naturally occurring plant, comprising genetically modifying cells of the plant to

express a PKS system comprising at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The domain of the PUFA PKS system is encoded by any of the nucleic acid sequences described above.

Yet another embodiment of the present invention relates to a method to modify an endproduct containing at least one fatty acid, comprising adding to the endproduct an oil produced by a recombinant host cell that expresses at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The domain of a PUFA PKS system is encoded by any of the nucleic acid sequences described above. In one aspect, the endproduct is selected from the group consisting of a dietary supplement, a food product, a pharmaceutical formulation, a humanized animal milk, and an infant formula. A pharmaceutical formulation can include, but is not limited to: an anti-inflammatory formulation, a chemotherapeutic agent, an active excipient, an osteoporosis drug, an anti-depressant, an anti-convulsant, an anti-*Heliobactor pylori* drug, a drug for treatment of neurodegenerative disease, a drug for treatment of degenerative liver disease, an antibiotic, and a cholesterol lowering formulation. In one aspect, the endproduct is used to treat a condition selected from the group consisting of: chronic inflammation, acute inflammation, gastrointestinal disorder, cancer, cachexia, cardiac restenosis, neurodegenerative disorder, degenerative disorder of the liver, blood lipid disorder, osteoporosis, osteoarthritis, autoimmune disease, preeclampsia, preterm birth, age related maculopathy, pulmonary disorder, and peroxisomal disorder.

Yet another embodiment of the present invention relates to a method to produce a humanized animal milk, comprising genetically modifying milk-producing cells of a milk-producing animal with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The domain of the PUFA PKS system is encoded by any of the nucleic acid sequences described above.

Yet another embodiment of the present invention relates to a method produce a recombinant microbe, comprising genetically modifying microbial cells to express at least one recombinant nucleic acid molecule comprising a comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The domain of the PUFA PKS system is encoded by any of the nucleic acid sequences described above.

Yet another embodiment of the present invention relates to a recombinant host cell which has been modified to express a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the PKS catalyzes both iterative and non-iterative enzymatic reactions. The PUFA PKS system comprises: (a) at least two enoyl ACP-reductase (ER) domains; (b) at least six acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. In one aspect, the PUFA PKS system is a eukaryotic PUFA PKS system. In another aspect, the PUFA PKS system is an algal PUFA PKS system, and preferably a Thraustochytriales PUFA PKS system, which can include, but is not limited to, a *Schizochytrium* PUFA PKS system or a *Thraustochytrium* PUFA PKS system.

In this embodiment, the PUFA PKS system can be expressed in a prokaryotic host cell or in a eukaryotic host cell. In one aspect, the host cell is a plant cell. Accordingly, one embodiment of the invention is a method to produce a product containing at least one PUFA, comprising growing a plant comprising such a plant cell under conditions effective to produce the product. The host cell is a microbial cell and in this case, one embodiment of the present invention is a method to produce a product containing at least one PUFA, comprising culturing a culture containing such a microbial cell under conditions effective to produce the product. In one aspect, the PKS system catalyzes the direct production of triglycerides.

Yet another embodiment of the present invention relates to a genetically modified microorganism comprising a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the PKS catalyzes both iterative and non-iterative enzymatic reactions. The PUFA PKS system comprises: (a) at least two enoyl ACP-reductase (ER) domains; (b) at least six acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. The genetic modification affects the activity of the PUFA PKS system. In one aspect of this embodiment, the microorganism is a eukaryotic microorganism.

Yet another embodiment of the present invention relates to a recombinant host cell which has been modified to express a non-bacterial polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the non-bacterial PUFA PKS catalyzes both iterative and non-iterative enzymatic reactions. The non-bacterial PUFA PKS system comprises: (a) at least one enoyl ACP-reductase (ER) domain; (b) multiple acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** is a graphical representation of the domain structure of the *Schizochytrium* PUFA PKS system.

FIG. **2** shows a comparison of PKS domains from *Schizochytrium* and *Shewanella.*

FIG. **3** shows a comparison of PKS domains from *Schizochytrium* and a related PKS system from *Nostoc* whose product is a long chain fatty acid that does not contain any double bonds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally relates to non-bacterial derived polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) systems, to genetically modified organisms comprising non-bacterial PUFA PKS systems, to methods of making and using such systems for the production of products of interest, including bioactive molecules, and to novel methods for identifying new eukaryotic microorganisms having such a PUFA PKS system. As used herein, a PUFA PKS system generally has the following identifying features: (1) it produces PUFAs as a natural product of the system; and (2) it

comprises several multifunctional proteins assembled into a complex that conducts both iterative processing of the fatty acid chain as well non-iterative processing, including trans-cis isomerization and enoyl reduction reactions in selected cycles (See FIG. **1**, for example).

More specifically, first, a PUFA PKS system that forms the basis of this invention produces polyunsaturated fatty acids (PUFAs) as products (i.e., an organism that endogenously (naturally) contains such a PKS system makes PUFAs using this system). The PUFAs referred to herein are preferably polyunsaturated fatty acids with a carbon chain length of at least 16 carbons, and more preferably at least 18 carbons, and more preferably at least 20 carbons, and more preferably 22 or more carbons, with at least 3 or more double bonds, and preferably 4 or more, and more preferably 5 or more, and even more preferably 6 or more double bonds, wherein all double bonds are in the cis configuration. It is an object of the present invention to find or create via genetic manipulation or manipulation of the endproduct, PKS systems which produce polyunsaturated fatty acids of desired chain length and with desired numbers of double bonds. Examples of PUFAs include, but are not limited to, DHA (docosahexaenoic acid (C22:6, ω-3)), DPA (docosapentaenoic acid (C22:5, ω-6)), and EPA (eicosapentaenoic acid (C20:5, ω-3)).

Second, the PUFA PKS system described herein incorporates both iterative and non-iterative reactions, which distinguish the system from previously described PKS systems (e.g., type I, type II or modular). More particularly, the PUFA PKS system described herein contains domains that appear to function during each cycle as well as those which appear to function during only some of the cycles. A key aspect of this may be related to the domains showing homology to the bacterial Fab A enzymes. For example, the Fab A enzyme of *E. coli* has been shown to possess two enzymatic activities. It possesses a dehydration activity in which a water molecule (H_2O) is abstracted from a carbon chain containing a hydroxy group, leaving a trans double bond in that carbon chain. In addition, it has an isomerase activity in which the trans double bond is converted to the cis configuration. This isomerization is accomplished in conjunction with a migration of the double bond position to adjacent carbons. In PKS (and FAS) systems, the main carbon chain is extended in 2 carbon increments. One can therefore predict the number of extension reactions required to produce the PUFA products of these PKS systems. For example, to produce DHA (C22:6, all cis) requires 10 extension reactions. Since there are only 6 double bonds in the end product, it means that during some of the reaction cycles, a double bond is retained (as a cis isomer), and in others, the double bond is reduced prior to the next extension.

Before the discovery of a PUFA PKS system in marine bacteria (see U.S. Pat. No. 6,140,486), PKS systems were not known to possess this combination of iterative and selective enzymatic reactions, and they were not thought of as being able to produce carbon-carbon double bonds in the cis configuration. However, the PUFA PKS system described by the present invention has the capacity to introduce cis double bonds and the capacity to vary the reaction sequence in the cycle.

Therefore, the present inventors propose to use these features of the PUFA PKS system to produce a range of bioactive molecules that could not be produced by the previously described (Type II, Type I and modular) PKS systems. These bioactive molecules include, but are limited to, polyunsaturated fatty acids (PUFAs), antibiotics or other bioactive compounds, many of which will be discussed below. For example, using the knowledge of the PUFA PKS gene structures described herein, any of a number of methods can be used to alter the PUFA PKS genes, or combine portions of these genes with other synthesis systems, including other PKS systems, such that new products are produced. The inherent ability of this particular type of system to do both iterative and selective reactions will enable this system to yield products that would not be found if similar methods were applied to other types of PKS systems.

In one embodiment, a PUFA PKS system according to the present invention comprises at least the following biologically active domains: (a) at least two enoyl ACP-reductase (ER) domains; (b) at least six acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. The functions of these domains are generally individually known in the art and will be described in detail below with regard to the PUFA PKS system of the present invention.

In another embodiment, the PUFA PKS system comprises at least the following biologically active domains: (a) at least one enoyl ACP-reductase (ER) domain; (b) multiple acyl carrier protein (ACP) domains (at least four, and preferably at least five, and more preferably at least six, and even more preferably seven, eight, nine, or more than nine); (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. Preferably, such a PUFA PKS system is a non-bacterial PUFA-PKS system.

In one embodiment, a PUFA PKS system of the present invention is a non-bacterial PUFA PKS system. In other words, in one embodiment, the PUFA PKS system of the present invention is isolated from an organism that is not a bacteria, or is a homologue of or derived from a PUFA PKS system from an organism that is not a bacteria, such as a eukaryote or an archaebacterium. Eukaryotes are separated from prokaryotes based on the degree of differentiation of the cells. The higher group with more differentiation is called eukaryotic. The lower group with less differentiated cells is called prokaryotic. In general, prokaryotes do no possess a nuclear membrane, do not exhibit mitosis during cell division, have only one chromosome, their cytoplasm contains 70S ribosomes, they do not possess any mitochondria, endoplasmic reticulum, chloroplasts, lysosomes or golgi apparatus, their flagella (if present) consists of a single fibril. In contrast eukaryotes have a nuclear membrane, they do exhibit mitosis during cell division, they have many chromosomes, their cytoplasm contains 80S ribosomes, they do possess mitochondria, endoplasmic reticulum, chloroplasts (in algae), lysosomes and golgi apparatus, and their flagella (if present) consists of many fibrils. In general, bacteria are prokaryotes, while algae, fungi, protist, protozoa and higher plants are eukaryotes. The PUFA PKS systems of the marine bacteria (e.g., *Shewanella* and *Vibrio marinus*) are not the basis of the present invention, although the present invention does contemplate the use of domains from these bacterial PUFA PKS systems in conjunction with domains from the non-bacterial PUFA PKS systems of the present invention. For example, according to the present invention, genetically modified organisms can be produced which incorporate non-bacterial PUFA PKS functional domains with bacteria PUFA

PKS functional domains, as well as PKS functional domains or proteins from other PKS systems (type I, type II, modular) or FAS systems.

Schizochytrium is a Thraustochytrid marine microorganism that accumulates large quantities of triacylglycerols rich in DHA and docosapentaenoic acid (DPA; 22:5 ω-6); e.g., 30% DHA+DPA by dry weight (Barclay et al., *J. Appl. Phycol.* 6, 123 (1994)). In eukaryotes that synthesize 20- and 22-carbon PUFAs by an elongation/desaturation pathway, the pools of 18-, 20- and 22-carbon intermediates are relatively large so that in vivo labeling experiments using [^{14}C]-acetate reveal clear precursor-product kinetics for the predicted intermediates (Gellerman et al., *Biochim. Biophys. Acta* 573:23 (1979)). Furthermore, radiolabeled intermediates provided exogenously to such organisms are converted to the final PUFA products. The present inventors have shown that [1-^{14}C]-acetate was rapidly taken up by *Schizochytrium* cells and incorporated into fatty acids, but at the shortest labeling time (1 min), DHA contained 31% of the label recovered in fatty acids, and this percentage remained essentially unchanged during the 10-15 min of [^{14}C]-acetate incorporation and the subsequent 24 hours of culture growth (See Example 3). Similarly, DPA represented 10% of the label throughout the experiment. There is no evidence for a precursor-product relationship between 16- or 18-carbon fatty acids and the 22-carbon polyunsaturated fatty acids. These results are consistent with rapid synthesis of DHA from [^{14}C]-acetate involving very small (possibly enzyme-bound) pools of intermediates. A cell-free homogenate derived from *Schizochytrium* cultures incorporated [1-^{14}C]-malonyl-CoA into DHA, DPA, and saturated fatty acids. The same biosynthetic activities were retained by a 100,000×g supernatant fraction but were not present in the membrane pellet. Thus, DHA and DPA synthesis in *Schizochytrium* does not involve membrane-bound desaturases or fatty acid elongation enzymes like those described for other eukaryotes (Parker-Barnes et al., 2000, supra; Shanklin et al., 1998, supra). These fractionation data contrast with those obtained from the *Shewanella* enzymes (See Metz et al., 2001, supra) and may indicate use of a different (soluble) acyl acceptor molecule, such as CoA, by the *Schizochytrium* enzyme.

In copending U.S. application Ser. No. 09/231,899, a cDNA library from *Schizochytrium* was constructed and approximately 8,000 random clones (ESTs) were sequenced. Within this dataset, only one moderately expressed gene (0.3% of all sequences) was identified as a fatty acid desaturase, although a second putative desaturase was represented by a single clone (0.01%). By contrast, sequences that exhibited homology to 8 of the 11 domains of the *Shewanella* PKS genes shown in FIG. **2** were all identified at frequencies of 0.2-0.5%. In U.S. application Ser. No. 09/231,899, several cDNA clones showing homology to the *Shewanella* PKS genes were sequenced, and various clones were assembled into nucleic acid sequences representing two partial open reading frames and one complete open reading frame. Nucleotides 390-4443 of the cDNA sequence containing the first partial open reading frame described in U.S. application Ser. No. 09/231,899 (denoted therein as SEQ ID NO:69) match nucleotides 4677-8730 (plus the stop codon) of the sequence denoted herein as OrfA (SEQ ID NO:1). Nucleotides 1-4876 of the cDNA sequence containing the second partial open reading frame described in U.S. application Ser. No. 09/231,899 (denoted therein as SEQ ID NO:71) matches nucleotides 1311-6177 (plus the stop codon) of the sequence denoted herein as OrfB (SEQ ID NO:3). Nucleotides 145-4653 of the cDNA sequence containing the complete open reading frame described in U.S. application Ser. No. 09/231,899 (denoted therein as SEQ ID NO:76 and incorrectly designated as a partial open reading frame) match the entire sequence (plus the stop codon) of the sequence denoted herein as OrfC (SEQ ID NO:5).

Further sequencing of cDNA and genomic clones by the present inventors allowed the identification of the full-length genomic sequence of each of OrfA, OrfB and OrfC and the complete identification of the domains with homology to those in *Shewanella* (see FIG. **2**). It is noted that in *Schizochytrium*, the genomic DNA and cDNA are identical, due to the lack of introns in the organism genome, to the best of the present inventors' knowledge. Therefore, reference to a nucleotide sequence from *Schizochytrium* can refer to genomic DNA or cDNA. Based on the comparison of the *Schizochytrium* PKS domains to *Shewanella*, clearly, the *Schizochytrium* genome encodes proteins that are highly similar to the proteins in *Shewanella* that are capable of catalyzing EPA synthesis. The proteins in *Schizochytrium* constitute a PUFA PKS system that catalyzes DHA and DPA synthesis. As discussed in detail herein, simple modification of the reaction scheme identified for *Shewanella* will allow for DHA synthesis in *Schizochytrium*. The homology between the prokaryotic *Shewanella* and eukaryotic *Schizochytrium* genes suggests that the PUFA PKS has undergone lateral gene transfer.

FIG. **1** is a graphical representation of the three open reading frames from the *Schizochytrium* PUFA PKS system, and includes the domain structure of this PUFA PKS system. As described in Example 1 below, the domain structure of each open reading frame is as follows:

Open Reading Frame A (OrfA):

The complete nucleotide sequence for OrfA is represented herein as SEQ ID NO:1. Nucleotides 4677-8730 of SEQ ID NO:1 correspond to nucleotides 390-4443 of the sequence denoted as SEQ ID NO:69 in U.S. application Ser. No. 09/231,899. Therefore, nucleotides 1-4676 of SEQ ID NO: 1 represent additional sequence that was not disclosed in U.S. application Ser. No. 09/231,899. This novel region of SEQ ID NO:1 encodes the following domains in OrfA: (1) the ORFA-KS domain; (2) the ORFA-MAT domain; and (3) at least a portion of the ACP domain region (e.g., at least ACP domains 1-4). It is noted that nucleotides 1-389 of SEQ ID NO:69 in U.S. application Ser. No. 09/231,899 do not match with the 389 nucleotides that are upstream of position 4677 in SEQ ID NO:1 disclosed herein. Therefore, positions 1-389 of SEQ ID NO:69 in U.S. application Ser. No. 09/231,899 appear to be incorrectly placed next to nucleotides 390-4443 of that sequence. Most of these first 389 nucleotides (about positions 60-389) are a match with an upstream portion of OrfA (SEQ ID NO:1) of the present invention and therefore, it is believed that an error occurred in the effort to prepare the contig of the cDNA constructs in U.S. application Ser. No. 09/231,899. The region in which the alignment error occurred in U.S. application Ser. No. 09/231,899 is within the region of highly repetitive sequence (i.e., the ACP region, discussed below), which probably created some confusion in the assembly of that sequence from various cDNA clones.

OrfA is a 8730 nucleotide sequence (not including the stop codon) which encodes a 2910 amino acid sequence, represented herein as SEQ ID NO:2. Within OrfA are twelve domains: (a) one β-keto acyl-ACP synthase (KS) domain; (b) one malonyl-CoA:ACP acyltransferase (MAT) domain; (c) nine acyl carrier protein (ACP) domains; and (d) one ketoreductase (KR) domain.

The nucleotide sequence for OrfA has been deposited with GenBank as Accession No. AF378327 (amino acid sequence

Accession No. AAK728879). OrfA was compared with known sequences in a standard BLAST search (BLAST 2.0 Basic BLAST homology search using blastp for amino acid searches, blastn for nucleic acid searches, and blastX for nucleic acid searches and searches of the translated amino acid sequence in all 6 open reading frames with standard default parameters, wherein the query sequence is filtered for low complexity regions by default (described in Altschul, S. F., Madden, T. L., Schääffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402, incorporated herein by reference in its entirety)). At the nucleic acid level, OrfA has no significant homology to any known nucleotide sequence. At the amino acid level, the sequences with the greatest degree of homology to ORFA were: *Nostoc* sp. 7120 heterocyst glycolipid synthase (Accession No. NC__003272), which was 42% identical to ORFA over 1001 amino acid residues; and *Moritella marinus* (*Vibrio marinus*) ORF8 (Accession No. AB025342), which was 40% identical to ORFA over 993 amino acid residues.

The first domain in OrfA is a KS domain, also referred to herein as ORFA-KS. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 40 of SEQ ID NO:1 (OrfA) to an ending point of between about positions 1428 and 1500 of SEQ ID NO:1. The nucleotide sequence containing the sequence encoding the ORFA-KS domain is represented herein as SEQ ID NO:7 (positions 1-1500 of SEQ ID NO:1). The amino acid sequence containing the KS domain spans from a starting point of between about positions 1 and 14 of SEQ ID NO:2 (ORFA) to an ending point of between about positions 476 and 500 of SEQ ID NO:2. The amino acid sequence containing the ORFA-KS domain is represented herein as SEQ ID NO:8 (positions 1-500 of SEQ ID NO:2). It is noted that the ORFA-KS domain contains an active site motif: DXAC* (*acyl binding site C_{215}).

According to the present invention, a domain or protein having 3-keto acyl-ACP synthase (KS) biological activity (function) is characterized as the enzyme that carries out the initial step of the FAS (and PKS) elongation reaction cycle. The acyl group destined for elongation is linked to a cysteine residue at the active site of the enzyme by a thioester bond. In the multi-step reaction, the acyl-enzyme undergoes condensation with malonyl-ACP to form -keto acyl-ACP, CO_2 and free enzyme. The KS plays a key role in the elongation cycle and in many systems has been shown to possess greater substrate specificity than other enzymes of the reaction cycle. For example, *E. coli* has three distinct KS enzymes—each with its own particular role in the physiology of the organism (Magnuson et al., *Microbiol. Rev.* 57, 522 (1993)). The two KS domains of the PUFA-PKS systems could have distinct roles in the PUFA biosynthetic reaction sequence.

As a class of enzymes, KS's have been well characterized. The sequences of many verified KS genes are know, the active site motifs have been identified and the crystal structures of several have been determined. Proteins (or domains of proteins) can be readily identified as belonging to the KS family of enzymes by homology to known KS sequences. The second domain in OrfA is a MAT domain, also referred to herein as ORFA-MAT. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1723 and 1798 of SEQ ID NO:1 (OrfA) to an ending point of between about positions 2805 and 3000 of SEQ ID NO:1. The nucleotide sequence containing the sequence encoding the ORFA-MAT domain is represented herein as SEQ ID NO:9 (positions 1723-3000 of SEQ ID NO:1). The amino acid sequence containing the MAT domain spans from a starting point of between about positions 575 and 600 of SEQ ID NO:2 (ORFA) to an ending point of between about positions 935 and 1000 of SEQ ID NO:2. The amino acid sequence containing the ORFA-MAT domain is represented herein as SEQ ID NO:10 (positions 575-1000 of SEQ ID NO:2). It is noted that the ORFA-MAT domain contains an active site motif: GHS*XG (*acyl binding site S_{706}), represented herein as SEQ ID NO:11.

According to the present invention, a domain or protein having malonyl-CoA:ACP acyltransferase (MAT) biological activity (function) is characterized as one that transfers the malonyl moiety from malonyl-CoA to ACP. In addition to the active site motif (GxSxG), these enzymes possess an extended motif ® and Q amino acids in key positions) that identifies them as MAT enzymes (in contrast to the AT domain of *Schizochytrium* Orf B). In some PKS systems (but not the PUFA PKS domain) MAT domains will preferentially load methyl- or ethyl-malonate on to the ACP group (from the corresponding CoA ester), thereby introducing branches into the linear carbon chain. MAT domains can be recognized by their homology to known MAT sequences and by their extended motif structure.

Domains 3-11 of OrfA are nine tandem ACP domains, also referred to herein as ORFA-ACP (the first domain in the sequence is ORFA-ACP 1, the second domain is ORFA-ACP2, the third domain is ORFA-ACP3, etc.). The first ACP domain, ORFA-ACP1, is contained within the nucleotide sequence spanning from about position 3343 to about position 3600 of SEQ ID NO: 1 (OrfA). The nucleotide sequence containing the sequence encoding the ORFA-ACP1 domain is represented herein as SEQ ID NO: 12 (positions 3343-3600 of SEQ ID NO: 1). The amino acid sequence containing the first ACP domain spans from about position 1115 to about position 1200 of SEQ ID NO:2. The amino acid sequence containing the ORFA-ACP1 domain is represented herein as SEQ ID NO: 13 (positions 1115-1200 of SEQ ID NO:2). It is noted that the ORFA-ACP1 domain contains an active site motif: LGIDS* (*pantetheine binding motif S_{1157}), represented herein by SEQ ID NO:14.

The nucleotide and amino acid sequences of all nine ACP domains are highly conserved and therefore, the sequence for each domain is not represented herein by an individual sequence identifier. However, based on the information disclosed herein, one of skill in the art can readily determine the sequence containing each of the other eight ACP domains (see discussion below).

All nine ACP domains together span a region of OrfA of from about position 3283 to about position 6288 of SEQ ID NO:1, which corresponds to amino acid positions of from about 1095 to about 2096 of SEQ ID NO:2. The nucleotide sequence for the entire ACP region containing all nine domains is represented herein as SEQ ID NO:16. The region represented by SEQ ID NO:16 includes the linker segments between individual ACP domains. The repeat interval for the nine domains is approximately every 330 nucleotides of SEQ ID NO:16 (the actual number of amino acids measured between adjacent active site serines ranges from 104 to 116 amino acids). Each of the nine ACP domains contains a pantetheine binding motif LGIDS* (represented herein by SEQ ID NO:14), wherein S* is the pantetheine binding site serine (S). The pantetheine binding site serine (S) is located near the center of each ACP domain sequence. At each end of the ACP domain region and between each ACP domain is a region that is highly enriched for proline (P) and alanine (A), which is believed to be a linker region. For example, between ACP domains 1 and 2 is the sequence: APAPVKAAA-

PAAPVASAPAPA, represented herein as SEQ ID NO:15. The locations of the active site serine residues (i.e., the pantetheine binding site) for each of the nine ACP domains, with respect to the amino acid sequence of SEQ ID NO:2, are as follows: ACP1=S_{1157}; ACP2=S_{1266}; ACP3=S_{1377}; ACP4=S_{1488}; ACP5=S_{1604}; ACP6=S_{1715}; ACP7=S_{1819}; ACP8=S_{1930}; and ACP9=S_{2034}. Given that the average size of an ACP domain is about 85 amino acids, excluding the linker, and about 110 amino acids including the linker, with the active site serine being approximately in the center of the domain, one of skill in the art can readily determine the positions of each of the nine ACP domains in OrfA.

According to the present invention, a domain or protein having acyl carrier protein (ACP) biological activity (function) is characterized as being small polypeptides (typically, 80 to 100 amino acids long), that function as carriers for growing fatty acyl chains via a thioester linkage to a covalently bound co-factor of the protein. They occur as separate units or as domains within larger proteins. ACPs are converted from inactive apo-forms to functional holo-forms by transfer of the phosphopantetheinyl moeity of CoA to a highly conserved serine residue of the ACP. Acyl groups are attached to ACP by a thioester linkage at the free terminus of the phosphopantetheinyl moiety. ACPs can be identified by labeling with radioactive pantetheine and by sequence homology to known ACPs. The presence of variations of the above mentioned motif (LGIDS*) is also a signature of an ACP.

Domain 12 in OrfA is a KR domain, also referred to herein as ORFA-KR. This domain is contained within the nucleotide sequence spanning from a starting point of about position 6598 of SEQ ID NO:1 to an ending point of about position 8730 of SEQ ID NO:1.

The nucleotide sequence containing the sequence encoding the ORFA-KR domain is represented herein as SEQ ID NO:17 (positions 6598-8730 of SEQ ID NO:1). The amino acid sequence containing the KR domain spans from a starting point of about position 2200 of SEQ ID NO:2 (ORFA) to an ending point of about position 2910 of SEQ ID NO:2. The amino acid sequence containing the ORFA-KR domain is represented herein as SEQ ID NO:18 (positions 2200-2910 of SEQ ID NO:2). Within the KR domain is a core region with homology to short chain aldehyde-dehydrogenases (KR is a member of this family). This core region spans from about position 7198 to about position 7500 of SEQ ID NO:1, which corresponds to amino acid positions 2400-2500 of SEQ ID NO:2.

According to the present invention, a domain or protein having ketoreductase activity, also referred to as 3-ketoacyl-ACP reductase (KR) biological activity (function), is characterized as one that catalyzes the pyridine-nucleotide-dependent reduction of 3-keto acyl forms of ACP. It is the first reductive step in the de novo fatty acid biosynthesis elongation cycle and a reaction often performed in polyketide biosynthesis. Significant sequence similarity is observed with one family of enoyl ACP reductases (ER), the other reductase of FAS (but not the ER family present in the PUFA PKS system), and the short-chain alcohol dehydrogenase family. Pfam analysis of the PUFA PKS region indicated above reveals the homology to the short-chain alcohol dehydrogenase family in the core region. Blast analysis of the same region reveals matches in the core area to known KR enzymes as well as an extended region of homology to domains from the other characterized PUFA PKS systems.

Open Reading Frame B (OrfB):

The complete nucleotide sequence for OrfB is represented herein as SEQ ID NO:3. Nucleotides 1311-4242 and 4244-6177 of SEQ ID NO:3 correspond to nucleotides 1-2932 and 2934-4867 of the sequence denoted as SEQ ID NO:71 in U.S. application Ser. No. 09/231,899 (The cDNA sequence in U.S. application Ser. No. 09/231,899 contains about 345 additional nucleotides beyond the stop codon, including a polyA tail). Therefore, nucleotides 1-1310 of SEQ ID NO:1 represent additional sequence that was not disclosed in U.S. application Ser. No. 09/231,899. This novel region of SEQ ID NO:3 contains most of the KS domain encoded by OrfB.

OrfB is a 6177 nucleotide sequence (not including the stop codon) which encodes a 2059 amino acid sequence, represented herein as SEQ ID NO:4. Within OrfB are four domains: (a) one β-keto acyl-ACP synthase (KS) domain; (b) one chain length factor (CLF) domain; (c) one acyl transferase (AT) domain; and, (d) one enoyl ACP-reductase (ER) domain.

The nucleotide sequence for OrfB has been deposited with GenBank as Accession No. AF378328 (amino acid sequence Accession No. AAK728880). OrfB was compared with known sequences in a standard BLAST search as described above. At the nucleic acid level, OrfB has no significant homology to any known nucleotide sequence. At the amino acid level, the sequences with the greatest degree of homology to ORFB were: *Shewanella* sp. hypothetical protein (Accession No. U73935), which was 53% identical to ORFB over 458 amino acid residues; *Moritella marinus* (*Vibrio marinus*) ORF11 (Accession No. AB025342), which was 53% identical to ORFB over 460 amino acid residues; *Photobacterium* profundum omega-3 polyunsaturated fatty acid synthase PfaD (Accession No. AF409100), which was 52% identical to ORFB over 457 amino acid residues; and *Nostoc* sp. 7120 hypothetical protein (Accession No. NC_003272), which was 53% identical to ORFB over 430 amino acid residues.

The first domain in OrfB is a KS domain, also referred to herein as ORFB-KS. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 43 of SEQ ID NO:3 (OrfB) to an ending point of between about positions 1332 and 1350 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-KS domain is represented herein as SEQ ID NO:19 (positions 1-1350 of SEQ ID NO:3). The amino acid sequence containing the KS domain spans from a starting point of between about positions 1 and 15 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 444 and 450 of SEQ ID NO:4. The amino acid sequence containing the ORFB-KS domain is represented herein as SEQ ID NO:20 (positions 1-450 of SEQ ID NO:4). It is noted that the ORFB-KS domain contains an active site motif: DXAC* (*acyl binding site C_{196}). KS biological activity and methods of identifying proteins or domains having such activity is described above.

The second domain in OrfB is a CLF domain, also referred to herein as ORFB-CLF. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1378 and 1402 of SEQ ID NO:3 (OrfB) to an ending point of between about positions 2682 and 2700 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-CLF domain is represented herein as SEQ ID NO:21 (positions 1378-2700 of SEQ ID NO:3). The amino acid sequence containing the CLF domain spans from a starting point of between about positions 460 and 468 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 894 and 900 of SEQ ID NO:4. The amino acid sequence containing the ORFB-CLF domain is

represented herein as SEQ ID NO:22 (positions 460-900 of SEQ ID NO:4). It is noted that the ORFB-CLF domain contains a KS active site motif without the acyl-binding cysteine.

According to the present invention, a domain or protein is referred to as a chain length factor (CLF) based on the following rationale. The CLF was originally described as characteristic of Type II (dissociated enzymes) PKS systems and was hypothesized to play a role in determining the number of elongation cycles, and hence the chain length, of the end product. CLF amino acid sequences show homology to KS domains (and are thought to form heterodimers with a KS protein), but they lack the active site cysteine. CLF's role in PKS systems is currently controversial. New evidence (C. Bisang et al., *Nature* 401, 502 (1999)) suggests a role in priming (providing the initial acyl group to be elongated) the PKS systems. In this role the CLF domain is thought to decarboxylate malonate (as malonyl-ACP), thus forming an acetate group that can be transferred to the KS active site. This acetate therefore acts as the 'priming' molecule that can undergo the initial elongation (condensation) reaction. Homologues of the Type II CLF have been identified as 'loading' domains in some modular PKS systems. A domain with the sequence features of the CLF is found in all currently identified PUFA PKS systems and in each case is found as part of a multidomain protein.

The third domain in OrfB is an AT domain, also referred to herein as ORFB-AT. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 2701 and 3598 of SEQ ID NO:3 (OrfB) to an ending point of between about positions 3975 and 4200 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-AT domain is represented herein as SEQ ID NO:23 (positions 2701-4200 of SEQ ID NO:3). The amino acid sequence containing the AT domain spans from a starting point of between about positions 901 and 1200 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 1325 and 1400 of SEQ ID NO:4. The amino acid sequence containing the ORFB-AT domain is represented herein as SEQ ID NO:24 (positions 901-1400 of SEQ ID NO:4). It is noted that the ORFB-AT domain contains an active site motif of GxS*xG (*acyl binding site S_{1140}) that is characteristic of acyltransferse (AT) proteins.

An "acyltransferase" or "AT" refers to a general class of enzymes that can carry out a number of distinct acyl transfer reactions. The *Schizochytrium* domain shows good homology to a domain present in all of the other PUFA PKS systems currently examined and very weak homology to some acyltransferases whose specific functions have been identified (e.g. to malonyl-CoA:ACP acyltransferase, MAT). In spite of the weak homology to MAT, this AT domain is not believed to function as a MAT because it does not possess an extended motif structure characteristic of such enzymes (see MAT domain description, above). For the purposes of this disclosure, the functions of the AT domain in a PUFA PKS system include, but are not limited to: transfer of the fatty acyl group from the ORFA ACP domain(s) to water (i.e. a thioesterase—releasing the fatty acyl group as a free fatty acid), transfer of a fatty acyl group to an acceptor such as CoA, transfer of the acyl group among the various ACP domains, or transfer of the fatty acyl group to a lipophilic acceptor molecule (e.g. to lysophosphadic acid).

The fourth domain in OrfB is an ER domain, also referred to herein as ORFB-ER. This domain is contained within the nucleotide sequence spanning from a starting point of about position 4648 of SEQ ID NO:3 (OrfB) to an ending point of about position 6177 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-ER domain is represented herein as SEQ ID NO:25 (positions 4648-6177 of SEQ ID NO:3). The amino acid sequence containing the ER domain spans from a starting point of about position 1550 of SEQ ID NO:4 (ORFB) to an ending point of about position 2059 of SEQ ID NO:4. The amino acid sequence containing the ORFB-ER domain is represented herein as SEQ ID NO:26 (positions 1550-2059 of SEQ ID NO:4).

According to the present invention, this domain has enoyl reductase (ER) biological activity. The ER enzyme reduces the trans-double bond (introduced by the DH activity) in the fatty acyl-ACP, resulting in fully saturating those carbons. The ER domain in the PUFA-PKS shows homology to a newly characterized family of ER enzymes (Heath et al., *Nature* 406, 145 (2000)). Heath and Rock identified this new class of ER enzymes by cloning a gene of interest from *Streptococcus pneumoniae*, purifying a protein expressed from that gene, and showing that it had ER activity in an in vitro assay. The sequence of the *Schizochytrium* ER domain of OrfB shows homology to the *S. pneumoniae* ER protein. All of the PUFA PKS systems currently examined contain at least one domain with very high sequence homology to the *Schizochytrium* ER domain. The *Schizochytrium* PUFA PKS system contains two ER domains (one on OrfB and one on OrfC).

Open Reading Frame C (OrfC):

The complete nucleotide sequence for OrfC is represented herein as SEQ ID NO:5. Nucleotides 1-4506 of SEQ ID NO:5 (i.e., the entire open reading frame sequence, not including the stop codon) correspond to nucleotides 145-2768, 2770-2805, 2807-2817, and 2819-4653 of the sequence denoted as SEQ ID NO:76 in U.S. application Ser. No. 09/231,899 (The cDNA sequence in U.S. application Ser. No. 09/231,899 contains about 144 nucleotides upstream of the start codon for OrfC and about 110 nucleotides beyond the stop codon, including a polyA tail). OrfC is a 4506 nucleotide sequence (not including the stop codon) which encodes a 1502 amino acid sequence, represented herein as SEQ ID NO:6. Within OrfC are three domains: (a) two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; and (b) one enoyl ACP-reductase (ER) domain.

The nucleotide sequence for OrfC has been deposited with GenBank as Accession No. AF378329 (amino acid sequence Accession No. AAK728881). OrfC was compared with known sequences in a standard BLAST search as described above. At the nucleic acid level, OrfC has no significant homology to any known nucleotide sequence. At the amino acid level (Blastp), the sequences with the greatest degree of homology to ORFC were: *Moritella marinus* (*Vibrio marinus*) ORF11 (Accession No. ABO25342), which is 45% identical to ORFC over 514 amino acid residues, *Shewanella* sp. hypothetical protein 8 (Accession No. U73935), which is 49% identical to ORFC over 447 amino acid residues, *Nostoc* sp. hypothetical protein (Accession No. NC_003272), which is 49% identical to ORFC over 430 amino acid residues, and *Shewanella* sp. hypothetical protein 7 (Accession No. U73935), which is 37% identical to ORFC over 930 amino acid residues.

The first domain in OrfC is a DH domain, also referred to herein as ORFC-DH1. This is one of two DH domains in OrfC, and therefore is designated DH1. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 778 of SEQ ID NO:5 (OrfC) to an ending point of between about positions 1233 and 1350 of SEQ ID NO:5. The nucleotide sequence

containing the sequence encoding the ORFC-DH1 domain is represented herein as SEQ ID NO:27 (positions 1-1350 of SEQ ID NO:5). The amino acid sequence containing the DH1 domain spans from a starting point of between about positions 1 and 260 of SEQ ID NO:6 (ORFC) to an ending point of between about positions 411 and 450 of SEQ ID NO:6. The amino acid sequence containing the ORFC-DH1 domain is represented herein as SEQ ID NO:28 (positions 1-450 of SEQ ID NO:6).

The characteristics of both the DH domains (see below for DH 2) in the PUFA PKS systems have been described in the preceding sections. This class of enzyme removes HOH from a β-keto acyl-ACP and leaves a trans double bond in the carbon chain. The DH domains of the PUFA PKS systems show homology to bacterial DH enzymes associated with their FAS systems (rather than to the DH domains of other PKS systems). A subset of bacterial DH's, the FabA-like DH's, possesses cis-trans isomerase activity (Heath et al., *J. Biol. Chem.,* 271, 27795 (1996)). It is the homologies to the FabA-like DH's that indicate that one or both of the DH domains is responsible for insertion of the cis double bonds in the PUFA PKS products.

The second domain in OrfC is a DH domain, also referred to herein as ORFC-DH2. This is the second of two DH domains in OrfC, and therefore is designated DH2. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1351 and 2437 of SEQ ID NO:5 (OrfC) to an ending point of between about positions 2607 and 2847 of SEQ ID NO:5. The nucleotide sequence containing the sequence encoding the ORFC-DH2 domain is represented herein as SEQ ID NO:29 (positions 1351-2847 of SEQ ID NO:5). The amino acid sequence containing the DH2 domain spans from a starting point of between about positions 451 and 813 of SEQ ID NO:6 (ORFC) to an ending point of between about positions 869 and 949 of SEQ ID NO:6. The amino acid sequence containing the ORFC-DH2 domain is represented herein as SEQ ID NO:30 (positions 451-949 of SEQ ID NO:6). DH biological activity has been described above.

The third domain in OrfC is an ER domain, also referred to herein as ORFC-ER. This domain is contained within the nucleotide sequence spanning from a starting point of about position 2995 of SEQ ID NO:5 (OrfC) to an ending point of about position 4506 of SEQ ID NO:5. The nucleotide sequence containing the sequence encoding the ORFC-ER domain is represented herein as SEQ ID NO:31 (positions 2995-4506 of SEQ ID NO:5). The amino acid sequence containing the ER domain spans from a starting point of about position 999 of SEQ ID NO:6 (ORFC) to an ending point of about position 1502 of SEQ ID NO:6. The amino acid sequence containing the ORFC-ER domain is represented herein as SEQ ID NO:32 (positions 999-1502 of SEQ ID NO:6). ER biological activity has been described above.

One embodiment of the present invention relates to an isolated nucleic acid molecule comprising a nucleic acid sequence from a non-bacterial PUFA PKS system, a homologue thereof, a fragment thereof, and/or a nucleic acid sequence that is complementary to any of such nucleic acid sequences. In one aspect, the present invention relates to an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6, and biologically active fragments thereof; (b) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (c) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of said amino acid sequence of (a), wherein said amino acid sequence has a biological activity of at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system; (d) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to said amino acid sequence of (b), wherein said amino acid sequence has a biological activity of at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system; or (e) a nucleic acid sequence that is fully complementary to the nucleic acid sequence of (a), (b), (c), or (d). In a further embodiment, nucleic acid sequences including a sequence encoding the active site domains or other functional motifs described above for several of the PUFA PKS domains are encompassed by the invention.

According to the present invention, an amino acid sequence that has a biological activity of at least one domain of a PUFA PKS system is an amino acid sequence that has the biological activity of at least one domain of the PUFA PKS system described in detail herein, as exemplified by the *Schizochytrium* PUFA PKS system. The biological activities of the various domains within the *Schizochytrium* PUFA PKS system have been described in detail above. Therefore, an isolated nucleic acid molecule of the present invention can encode the translation product of any PUFA PKS open reading frame, PUFA PKS domain, biologically active fragment thereof, or any homologue of a naturally occurring PUFA PKS open reading frame or domain which has biological activity. A homologue of given protein or domain is a protein or polypeptide that has an amino acid sequence which differs from the naturally occurring reference amino acid sequence (i.e., of the reference protein or domain) in that at least one or a few, but not limited to one or a few, amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide or fragment), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidyl inositol). Preferred homologues of a PUFA PKS protein or domain are described in detail below. It is noted that homologues can include synthetically produced homologues, naturally occurring allelic variants of a given protein or domain, or homologous sequences from organisms other than the organism from which the reference sequence was derived.

In general, the biological activity or biological action of a protein or domain refers to any function(s) exhibited or performed by the protein or domain that is ascribed to the naturally occurring form of the protein or domain as measured or observed in vivo (i.e., in the natural physiological environment of the protein) or in vitro (i.e., under laboratory conditions). Biological activities of PUFA PKS systems and the individual proteins/domains that make up a PUFA PKS system have been described in detail elsewhere herein. Modifications of a protein or domain, such as in a homologue or mimetic (discussed below), may result in proteins or domains having the same biological activity as the naturally occurring protein or domain, or in proteins or domains having decreased or increased biological activity as compared to the naturally occurring protein or domain. Modifications which result in a decrease in expression or a decrease in the activity of the protein or domain, can be referred to as inactivation (complete or partial), down-regulation, or decreased action of a protein or domain. Similarly, modifications which result in an

increase in expression or an increase in the activity of the protein or domain, can be referred to as amplification, overproduction, activation, enhancement, up-regulation or increased action of a protein or domain. A functional domain of a PUFA PKS system is a domain (i.e., a domain can be a portion of a protein) that is capable of performing a biological function (i.e., has biological activity).

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation), its natural milieu being the genome or chromosome in which the nucleic acid molecule is found in nature. As such, "isolated" does not necessarily reflect the extent to which the nucleic acid molecule has been purified, but indicates that the molecule does not include an entire genome or an entire chromosome in which the nucleic acid molecule is found in nature. An isolated nucleic acid molecule can include a gene. An isolated nucleic acid molecule that includes a gene is not a fragment of a chromosome that includes such gene, but rather includes the coding region and regulatory regions associated with the gene, but no additional genes naturally found on the same chromosome. An isolated nucleic acid molecule can also include a specified nucleic acid sequence flanked by (i.e., at the 5' and/or the 3' end of the sequence) additional nucleic acids that do not normally flank the specified nucleic acid sequence in nature (i.e., heterologous sequences). Isolated nucleic acid molecule can include DNA, RNA (e.g., mRNA), or derivatives of either DNA or RNA (e.g., cDNA). Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a protein or domain of a protein.

Preferably, an isolated nucleic acid molecule of the present invention is produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications provide the desired effect on PUFA PKS system biological activity as described herein. Protein homologues (e.g., proteins encoded by nucleic acid homologues) have been discussed in detail above.

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid and/or by hybridization with a wild-type gene.

The minimum size of a nucleic acid molecule of the present invention is a size sufficient to form a probe or oligonucleotide primer that is capable of forming a stable hybrid (e.g., under moderate, high or very high stringency conditions) with the complementary sequence of a nucleic acid molecule useful in the present invention, or of a size sufficient to encode an amino acid sequence having a biological activity of at least one domain of a PUFA PKS system according to the present invention. As such, the size of the nucleic acid molecule encoding such a protein can be dependent on nucleic acid composition and percent homology or identity between the nucleic acid molecule and complementary sequence as well as upon hybridization conditions per se (e.g., temperature, salt concentration, and formamide concentration). The minimal size of a nucleic acid molecule that is used as an oligonucleotide primer or as a probe is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecules are GC-rich and at least about 15 to about 18 bases in length if they are AT-rich. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule of the present invention, in that the nucleic acid molecule can include a sequence sufficient to encode a biologically active fragment of a domain of a PUFA PKS system, an entire domain of a PUFA PKS system, several domains within an open reading frame (Orf) of a PUFA PKS system, an entire Orf of a PUFA PKS system, or more than one Orf of a PUFA PKS system.

In one embodiment of the present invention, an isolated nucleic acid molecule comprises or consists essentially of a nucleic acid sequence selected from the group of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, or biologically active fragments thereof. In one aspect, the nucleic acid sequence is selected from the group of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, and SEQ ID NO:31. In one embodiment of the present invention, any of the above-described PUFA PKS amino acid sequences, as well as homologues of such sequences, can be produced with from at least one, and up to about 20, additional heterologous amino acids flanking each of the C- and/or N-terminal end of the given amino acid sequence. The resulting protein or polypeptide can be referred to as "consisting essentially of" a given amino acid sequence. According to the present invention, the heterologous amino acids are a sequence of amino acids that are not naturally found (i.e., not found in nature, in vivo) flanking the given amino acid sequence or which would not be encoded by the nucleotides that flank the naturally occurring nucleic acid sequence encoding the given amino acid sequence as it occurs in the gene, if such nucleotides in the naturally occurring sequence were translated using standard codon usage for the organism from which the given amino acid sequence is derived. Similarly, the phrase "consisting essentially of", when used with reference to a nucleic acid sequence herein, refers to a nucleic acid sequence encoding a given amino acid sequence that can be flanked by from at least one, and up to as many as about 60, additional heterologous nucleotides at each of the 5' and/or the 3' end of the nucleic acid sequence encoding the given amino acid sequence. The heterologous nucleotides are not naturally found (i.e., not found in nature, in vivo) flanking the nucleic acid sequence encoding the given amino acid sequence as it occurs in the natural gene.

The present invention also includes an isolated nucleic acid molecule comprising a nucleic acid sequence encoding an amino acid sequence having a biological activity of at least one domain of a PUFA PKS system. In one aspect, such a nucleic acid sequence encodes a homologue of any of the *Schizochytrium* PUFA PKS ORFs or domains, including: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32, wherein the homologue has a biological activity of at least one domain of a PUFA PKS system as described previously herein.

In one aspect of the invention, a homologue of a *Schizochytrium* PUFA PKS protein or domain encompassed by the present invention comprises an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence chosen from: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein said amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. In a further aspect, the amino acid sequence of the homologue is at least about 60% identical to at least about 600 consecutive amino acids, and more preferably to at least about 700 consecutive amino acids, and more preferably to at least about 800 consecutive amino acids, and more preferably to at least about 900 consecutive amino acids, and more preferably to at least about 1000 consecutive amino acids, and more preferably to at least about 1100 consecutive amino acids, and more preferably to at least about 1200 consecutive amino acids, and more preferably to at least about 1300 consecutive amino acids, and more preferably to at least about 1400 consecutive amino acids, and more preferably to at least about 1500 consecutive amino acids of any of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6, or to the full length of SEQ ID NO:6. In a further aspect, the amino acid sequence of the homologue is at least about 60% identical to at least about 1600 consecutive amino acids, and more preferably to at least about 1700 consecutive amino acids, and more preferably to at least about 1800 consecutive amino acids, and more preferably to at least about 1900 consecutive amino acids, and more preferably to at least about 2000 consecutive amino acids of any of SEQ ID NO:2 or SEQ ID NO:4, or to the full length of SEQ ID NO:4. In a further aspect, the amino acid sequence of the homologue is at least about 60% identical to at least about 2100 consecutive amino acids, and more preferably to at least about 2200 consecutive amino acids, and more preferably to at least about 2300 consecutive amino acids, and more preferably to at least about 2400 consecutive amino acids, and more preferably to at least about 2500 consecutive amino acids, and more preferably to at least about 2600 consecutive amino acids, and more preferably to at least about 2700 consecutive amino acids, and more preferably to at least about 2800 consecutive amino acids, and even more preferably, to the full length of SEQ ID NO:2.

In another aspect, a homologue of a *Schizochytrium* PUFA PKS protein or domain encompassed by the present invention comprises an amino acid sequence that is at least about 65% identical, and more preferably at least about 70% identical, and more preferably at least about 75% identical, and more preferably at least about 80% identical, and more preferably at least about 85% identical, and more preferably at least about 90% identical, and more preferably at least about 95% identical, and more preferably at least about 96% identical, and more preferably at least about 97% identical, and more preferably at least about 98% identical, and more preferably at least about 99% identical to an amino acid sequence chosen from: SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6, over any of the consecutive amino acid lengths described in the paragraph above, wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system.

In one aspect of the invention, a homologue of a *Schizochytrium* PUFA PKS protein or domain encompassed by the present invention comprises an amino acid sequence that is at least about 60% identical to an amino acid sequence chosen from: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32, wherein said amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. In a further aspect, the amino acid sequence of the homologue is at least about 65% identical, and more preferably at least about 70% identical, and more preferably at least about 75% identical, and more preferably at least about 80% identical, and more preferably at least about 85% identical, and more preferably at least about 90% identical, and more preferably at least about 95% identical, and more preferably at least about 96% identical, and more preferably at least about 97% identical, and more preferably at least about 98% identical, and more preferably at least about 99% identical to an amino acid sequence chosen from: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system.

According to the present invention, the term "contiguous" or "consecutive", with regard to nucleic acid or amino acid sequences described herein, means to be connected in an unbroken sequence. For example, for a first sequence to comprise 30 contiguous (or consecutive) amino acids of a second sequence, means that the first sequence includes an unbroken sequence of 30 amino acid residues that is 100% identical to an unbroken sequence of 30 amino acid residues in the second sequence. Similarly, for a first sequence to have "100% identity" with a second sequence means that the first sequence exactly matches the second sequence with no gaps between nucleotides or amino acids.

As used herein, unless otherwise specified, reference to a percent (%) identity refers to an evaluation of homology which is performed using: (1) a BLAST 2.0 Basic BLAST homology search using blastp for amino acid searches, blastn for nucleic acid searches, and blastX for nucleic acid searches and searches of translated amino acids in all 6 open reading frames, all with standard default parameters, wherein the query sequence is filtered for low complexity regions by default (described in Altschul, S. F., Madden, T. L., Schääffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402, incorporated herein by reference in its entirety); (2) a BLAST 2 alignment (using the parameters described below); (3) and/or PSI-BLAST with the standard default parameters (Position-Specific Iterated BLAST). It is noted that due to some differences in the standard parameters between BLAST 2.0 Basic BLAST and BLAST 2, two specific sequences might be recognized as having significant homology using the BLAST 2 program, whereas a search performed in BLAST 2.0 Basic BLAST using one of the sequences as the query sequence may not identify the second sequence in the top matches. In addition, PSI-BLAST provides an automated, easy-to-use version of a "profile" search, which is a sensitive way to look for sequence homologues. The program first performs a gapped BLAST database search. The PSI-BLAST

program uses the information from any significant alignments returned to construct a position-specific score matrix, which replaces the query sequence for the next round of database searching. Therefore, it is to be understood that percent identity can be determined by using any one of these programs.

Two specific sequences can be aligned to one another using BLAST 2 sequence as described in Tatusova and Madden, (1999), "Blast 2 sequences—a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.* 174: 247-250, incorporated herein by reference in its entirety. BLAST 2 sequence alignment is performed in blastp or blastn using the BLAST 2.0 algorithm to perform a Gapped BLAST search (BLAST 2.0) between the two sequences allowing for the introduction of gaps (deletions and insertions) in the resulting alignment. For purposes of clarity herein, a BLAST 2 sequence alignment is performed using the standard default parameters as follows.

For blastn, using 0 BLOSUM62 matrix:
- Reward for match = 1
- Penalty for mismatch = −2
- Open gap (5) and extension gap (2) penalties
- gap x_dropoff (50) expect (10) word size (11) filter (on)

For blastp, using 0 BLOSUM62 matrix:
- Open gap (11) and extension gap (1) penalties
- gap x_dropoff (50) expect (10) word size (3) filter (on).

In another embodiment of the invention, an amino acid sequence having the biological activity of at least one domain of a PUFA PKS system of the present invention includes an amino acid sequence that is sufficiently similar to a naturally occurring PUFA PKS protein or polypeptide that a nucleic acid sequence encoding the amino acid sequence is capable of hybridizing under moderate, high, or very high stringency conditions (described below) to (i.e., with) a nucleic acid molecule encoding the naturally occurring PUFA PKS protein or polypeptide (i.e., to the complement of the nucleic acid strand encoding the naturally occurring PUFA PKS protein or polypeptide). Preferably, an amino acid sequence having the biological activity of at least one domain of a PUFA PKS system of the present invention is encoded by a nucleic acid sequence that hybridizes under moderate, high or very high stringency conditions to the complement of a nucleic acid sequence that encodes a protein comprising an amino acid sequence represented by any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32. Methods to deduce a complementary sequence are known to those skilled in the art. It should be noted that since amino acid sequencing and nucleic acid sequencing technologies are not entirely error-free, the sequences presented herein, at best, represent apparent sequences of PUFA PKS domains and proteins of the present invention.

As used herein, hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. Sambrook et al., ibid., is incorporated by reference herein in its entirety (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are disclosed, for example, in Meinkoth et al., 1984, *Anal. Biochem.* 138, 267-284; Meinkoth et al., ibid., is incorporated by reference herein in its entirety.

More particularly, moderate stringency hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction (i.e., conditions permitting about 30% or less mismatch of nucleotides). High stringency hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 80% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction (i.e., conditions permitting about 20% or less mismatch of nucleotides). Very high stringency hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 90% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction (i.e., conditions permitting about 10% or less mismatch of nucleotides). As discussed above, one of skill in the art can use the formulae in Meinkoth et al., ibid. to calculate the appropriate hybridization and wash conditions to achieve these particular levels of nucleotide mismatch. Such conditions will vary, depending on whether DNA:RNA or DNA:DNA hybrids are being formed. Calculated melting temperatures for DNA:DNA hybrids are 10° C. less than for DNA:RNA hybrids. In particular embodiments, stringent hybridization conditions for DNA:DNA hybrids include hybridization at an ionic strength of 6×SSC (0.9 M Na^+) at a temperature of between about 20° C. and about 35° C. (lower stringency), more preferably, between about 28° C. and about 40° C. (more stringent), and even more preferably, between about 35° C. and about 45° C. (even more stringent), with appropriate wash conditions. In particular embodiments, stringent hybridization conditions for DNA:RNA hybrids include hybridization at an ionic strength of 6×SSC (0.9 M Na^+) at a temperature of between about 30° C. and about 45° C., more preferably, between about 38° C. and about 50° C., and even more preferably, between about 45° C. and about 55° C., with similarly stringent wash conditions. These values are based on calculations of a melting temperature for molecules larger than about 100 nucleotides, 0% formamide and a G+C content of about 40%. Alternatively, T_m can be calculated empirically as set forth in Sambrook et al., supra, pages 9.31 to 9.62. In general, the wash conditions should be as stringent as possible, and should be appropriate for the chosen hybridization conditions. For example, hybridization conditions can include a combination of salt and temperature conditions that are approximately 20-25° C. below the calculated T_m of a particular hybrid, and wash conditions typically include a combination of salt and temperature conditions that are approximately 12-20° C. below the calculated T_m of the particular hybrid. One example of hybridization conditions suitable for use with DNA:DNA hybrids includes a 2-24 hour hybridization in 6×SSC (50% formamide) at about 42° C., followed by washing steps that include one or more washes at room temperature in about 2×SSC, followed by additional washes at higher temperatures and lower ionic strength (e.g., at least one wash as about 37° C. in about 0.1×-0.5×SSC, followed by at least one wash at about 68° C. in about 0.1×-0.5×SSC).

Another embodiment of the present invention includes a recombinant nucleic acid molecule comprising a recombinant vector and a nucleic acid molecule comprising a nucleic acid sequence encoding an amino acid sequence having a

biological activity of at least one domain of a PUFA PKS system as described herein. Such nucleic acid sequences are described in detail above. According to the present invention, a recombinant vector is an engineered (i.e., artificially produced) nucleic acid molecule that is used as a tool for manipulating a nucleic acid sequence of choice and for introducing such a nucleic acid sequence into a host cell. The recombinant vector is therefore suitable for use in cloning, sequencing, and/or otherwise manipulating the nucleic acid sequence of choice, such as by expressing and/or delivering the nucleic acid sequence of choice into a host cell to form a recombinant cell. Such a vector typically contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid sequence to be cloned or delivered, although the vector can also contain regulatory nucleic acid sequences (e.g., promoters, untranslated regions) which are naturally found adjacent to nucleic acid molecules of the present invention or which are useful for expression of the nucleic acid molecules of the present invention (discussed in detail below). The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a plasmid. The vector can be maintained as an extrachromosomal element (e.g., a plasmid) or it can be integrated into the chromosome of a recombinant organism (e.g., a microbe or a plant). The entire vector can remain in place within a host cell, or under certain conditions, the plasmid DNA can be deleted, leaving behind the nucleic acid molecule of the present invention. The integrated nucleic acid molecule can be under chromosomal promoter control, under native or plasmid promoter control, or under a combination of several promoter controls. Single or multiple copies of the nucleic acid molecule can be integrated into the chromosome. A recombinant vector of the present invention can contain at least one selectable marker.

In one embodiment, a recombinant vector used in a recombinant nucleic acid molecule of the present invention is an expression vector. As used herein, the phrase "expression vector" is used to refer to a vector that is suitable for production of an encoded product (e.g., a protein of interest). In this embodiment, a nucleic acid sequence encoding the product to be produced (e.g., a PUFA PKS domain) is inserted into the recombinant vector to produce a recombinant nucleic acid molecule. The nucleic acid sequence encoding the protein to be produced is inserted into the vector in a manner that operatively links the nucleic acid sequence to regulatory sequences in the vector which enable the transcription and translation of the nucleic acid sequence within the recombinant host cell.

In another embodiment, a recombinant vector used in a recombinant nucleic acid molecule of the present invention is a targeting vector. As used herein, the phrase "targeting vector" is used to refer to a vector that is used to deliver a particular nucleic acid molecule into a recombinant host cell, wherein the nucleic acid molecule is used to delete or inactivate an endogenous gene within the host cell or microorganism (i.e., used for targeted gene disruption or knock-out technology). Such a vector may also be known in the art as a "knock-out" vector. In one aspect of this embodiment, a portion of the vector, but more typically, the nucleic acid molecule inserted into the vector (i.e., the insert), has a nucleic acid sequence that is homologous to a nucleic acid sequence of a target gene in the host cell (i.e., a gene which is targeted to be deleted or inactivated). The nucleic acid sequence of the vector insert is designed to bind to the target gene such that the target gene and the insert undergo homologous recombination, whereby the endogenous target gene is deleted, inactivated or attenuated (i.e., by at least a portion of the endogenous target gene being mutated or deleted).

Typically, a recombinant nucleic acid molecule includes at least one nucleic acid molecule of the present invention operatively linked to one or more transcription control sequences. As used herein, the phrase "recombinant molecule" or "recombinant nucleic acid molecule" primarily refers to a nucleic acid molecule or nucleic acid sequence operatively linked to a transcription control sequence, but can be used interchangeably with the phrase "nucleic acid molecule", when such nucleic acid molecule is a recombinant molecule as discussed herein. According to the present invention, the phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be expressed when transfected (i.e., transformed, transduced, transfected, conjugated or conduced) into a host cell. Transcription control sequences are sequences which control the initiation, elongation, or termination of transcription.

Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in a host cell or organism into which the recombinant nucleic acid molecule is to be introduced.

Recombinant nucleic acid molecules of the present invention can also contain additional regulatory sequences, such as translation regulatory sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell. In one embodiment, a recombinant molecule of the present invention, including those which are integrated into the host cell chromosome, also contains secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed protein to be secreted from the cell that produces the protein. Suitable signal segments include a signal segment that is naturally associated with the protein to be expressed or any heterologous signal segment capable of directing the secretion of the protein according to the present invention. In another embodiment, a recombinant molecule of the present invention comprises a leader sequence to enable an expressed protein to be delivered to and inserted into the membrane of a host cell. Suitable leader sequences include a leader sequence that is naturally associated with the protein, or any heterologous leader sequence capable of directing the delivery and insertion of the protein to the membrane of a cell.

The present inventors have found that the *Schizochytrium* PUFA PKS Orfs A and B are closely linked in the genome and region between the Orfs has been sequenced. The Orfs are oriented in opposite directions and 4244 base pairs separate the start (ATG) codons (i.e. they are arranged as follows: 3'OrfA5'-4244 bp-5'OrfB3'). Examination of the 4244 bp intergenic region did not reveal any obvious Orfs (no significant matches were found on a BlastX search). Both Orfs A and B are highly expressed in *Schizochytrium*, at least during the time of oil production, implying that active promoter elements are embedded in this intergenic region. These genetic elements are believed to have utility as a bi-directional promoter sequence for transgenic applications. For example, in a preferred embodiment, one could clone this region, place any genes of interest at each end and introduce the construct into *Schizochytrium* (or some other host in which the promoters can be shown to function). It is predicted that the regulatory elements, under the appropriate conditions, would provide for coordinated, high level expression of the two introduced genes. The complete nucleotide sequence

for the regulatory region containing *Schizochytrium* PUFA PKS regulatory elements (e.g., a promoter) is represented herein as SEQ ID NO:36.

In a similar manner, OrfC is highly expressed in *Schizochytrium* during the time of oil production and regulatory elements are expected to reside in the region upstream of its start codon. A region of genomic DNA upstream of OrfC has been cloned and sequenced and is represented herein as (SEQ ID NO:37). This sequence contains the 3886 nt immediately upstream of the OrfC start codon. Examination of this region did not reveal any obvious Orfs (i.e., no significant matches were found on a BlastX search). It is believed that regulatory elements contained in this region, under the appropriate conditions, will provide for high-level expression of a gene placed behind them. Additionally, under the appropriate conditions, the level of expression may be coordinated with genes under control of the A-B intergenic region (SEQ ID NO:36).

Therefore, in one embodiment, a recombinant nucleic acid molecule useful in the present invention, as disclosed herein, can include a PUFA PKS regulatory region contained within SEQ ID NO:36 and/or SEQ ID NO:37. Such a regulatory region can include any portion (fragment) of SEQ ID NO:36 and/or SEQ ID NO:37 that has at least basal PUFA PKS transcriptional activity.

One or more recombinant molecules of the present invention can be used to produce an encoded product (e.g., a PUFA PKS domain, protein, or system) of the present invention. In one embodiment, an encoded product is produced by expressing a nucleic acid molecule as described herein under conditions effective to produce the protein. A preferred method to produce an encoded protein is by transfecting a host cell with one or more recombinant molecules to form a recombinant cell. Suitable host cells to transfect include, but are not limited to, any bacterial, fungal (e.g., yeast), insect, plant or animal cell that can be transfected. Host cells can be either untransfected cells or cells that are already transfected with at least one other recombinant nucleic acid molecule.

According to the present invention, the term "transfection" is used to refer to any method by which an exogenous nucleic acid molecule (i.e., a recombinant nucleic acid molecule) can be inserted into a cell. The term "transformation" can be used interchangeably with the term "transfection" when such term is used to refer to the introduction of nucleic acid molecules into microbial cells, such as algae, bacteria and yeast. In microbial systems, the term "transformation" is used to describe an inherited change due to the acquisition of exogenous nucleic acids by the microorganism and is essentially synonymous with the term "transfection." However, in animal cells, transformation has acquired a second meaning which can refer to changes in the growth properties of cells in culture after they become cancerous, for example. Therefore, to avoid confusion, the term "transfection" is preferably used with regard to the introduction of exogenous nucleic acids into animal cells, and the term "transfection" will be used herein to generally encompass transfection of animal cells, plant cells and transformation of microbial cells, to the extent that the terms pertain to the introduction of exogenous nucleic acids into a cell. Therefore, transfection techniques include, but are not limited to, transformation, particle bombardment, electroporation, microinjection, lipofection, adsorption, infection and protoplast fusion.

It will be appreciated by one skilled in the art that use of recombinant DNA technologies can improve control of expression of transfected nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within the host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Additionally, the promoter sequence might be genetically engineered to improve the level of expression as compared to the native promoter. Recombinant techniques useful for controlling the expression of nucleic acid molecules include, but are not limited to, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules to correspond to the codon usage of the host cell, and deletion of sequences that destabilize transcripts.

General discussion above with regard to recombinant nucleic acid molecules and transfection of host cells is intended to be applied to any recombinant nucleic acid molecule discussed herein, including those encoding any amino acid sequence having a biological activity of at least one domain from a PUFA PKS, those encoding amino acid sequences from other PKS systems, and those encoding other proteins or domains.

This invention also relates to the use of a novel method to identify a microorganism that has a PUFA PKS system that is homologous in structure, domain organization and/or function to a *Schizochytrium* PUFA PKS system. In one embodiment, the microorganism is a non-bacterial microorganism, and preferably, the microorganism identified by this method is a eukaryotic microorganism. In addition, this invention relates to the microorganisms identified by such method and to the use of these microorganisms and the PUFA PKS systems from these microorganisms in the various applications for a PUFA PKS system (e.g., genetically modified organisms and methods of producing bioactive molecules) according to the present invention. The unique screening method described and demonstrated herein enables the rapid identification of new microbial strains containing a PUFA PKS system homologous to the *Schizochytrium* PUFA PKS system of the present invention. Applicants have used this method to discover and disclose herein that a *Thraustochytrium* microorganism contains a PUFA PKS system that is homologous to that found in *Schizochytrium*. This discovery is described in detail in Example 2 below.

Microbial organisms with a PUFA PKS system similar to that found in *Schizochytrium*, such as the *Thraustochytrium* microorganism discovered by the present inventors and described in Example 2, can be readily identified/isolated/screened by the following methods used separately or in any combination of these methods.

In general, the method to identify a non-bacterial microorganism that has a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system includes a first step of (a) selecting a microorganism that produces at least one PUFA; and a second step of (b) identifying a microorganism from (a) that has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation in the fermentation medium, as compared to production of PUFAs by said microorganism under dissolved oxygen conditions of greater than 5% of saturation, more preferably 10% of saturation, more preferably greater than 15% of saturation and more preferably greater than 20% of saturation in the fermentation medium. A microorganism that produces at least one PUFA and has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation is identified as a candidate for containing a

PUFA PKS system. Subsequent to identifying a microorganism that is a strong candidate for containing a PUFA PKS system, the method can include an additional step (c) of detecting whether the organism identified in step (b) comprises a PUFA PKS system.

In one embodiment of the present invention, step (b) is performed by culturing the microorganism selected for the screening process in low oxygen/anoxic conditions and aerobic conditions, and, in addition to measuring PUFA content in the organism, the fatty acid profile is determined, as well as fat content. By comparing the results under low oxygen/anoxic conditions with the results under aerobic conditions, the method provides a strong indication of whether the test microorganism contains a PUFA PKS system of the present invention. This preferred embodiment is described in detail below.

Initially, microbial strains to be examined for the presence of a PUFA PKS system are cultured under aerobic conditions to induce production of a large number of cells (microbial biomass). As one element of the identification process, these cells are then placed under low oxygen or anoxic culture conditions (e.g., dissolved oxygen less than about 5% of saturation, more preferably less than about 2%, even more preferably less than about 1%, and most preferably dissolved oxygen of about 0% of saturation in the culture medium) and allowed to grow for approximately another 24-72 hours. In this process, the microorganisms should be cultured at a temperature greater than about 15° C., and more preferably greater than about 20° C., and even more preferably greater than about 25° C., and even more preferably greater than 30° C. The low or anoxic culture environment can be easily maintained in culture chambers capable of inducing this type of atmospheric environment in the chamber (and thus in the cultures) or by culturing the cells in a manner that induces the low oxygen environment directly in the culture flask/vessel itself.

In a preferred culturing method, the microbes can be cultured in shake flasks which, instead of normally containing a small amount of culture medium—less than about 50% of total capacity and usually less than about 25% of total capacity—to keep the medium aerated as it is shaken on a shaker table, are instead filled to greater than about 50% of their capacity, and more preferably greater than about 60%, and most preferably greater than about 75% of their capacity with culture medium. High loading of the shake flask with culture medium prevents it from mixing very well in the flask when it is placed on a shaker table, preventing oxygen diffusion into the culture. Therefore as the microbes grow, they use up the existing oxygen in the medium and naturally create a low or no oxygen environment in the shake flask.

After the culture period, the cells are harvested and analyzed for content of bioactive compounds of interest (e.g., lipids), but most particularly, for compounds containing two or more unsaturated bonds, and more preferably three or more double bonds, and even more preferably four or more double bonds. For lipids, those strains possessing such compounds at greater than about 5%, and more preferably greater than about 10%, and more preferably greater than about 15%, and even more preferably greater than about 20% of the dry weight of the microorganism are identified as predictably containing a novel PKS system of the type described above. For other bioactive compounds, such as antibiotics or compounds that are synthesized in smaller amounts, those strains possessing such compounds at greater than about 0.5%, and more preferably greater than about 0.1%, and more preferably greater than about 0.25%, and more preferably greater than about 0.5%, and more preferably greater than about 0.75%, and more preferably greater than about 1%, and more preferably greater than about 2.5%, and more preferably greater than about 5% of the dry weight of the microorganism are identified as predictably containing a novel PKS system of the type described above.

Alternatively, or in conjunction with this method, prospective microbial strains containing novel PUFA PKS systems as described herein can be identified by examining the fatty acid profile of the strain (obtained by culturing the organism or through published or other readily available sources). If the microbe contains greater than about 30%, and more preferably greater than about 40%, and more preferably greater than about 45%, and even more preferably greater than about 50% of its total fatty acids as C14:0, C16:0 and/or C16:1, while also producing at least one long chain fatty acid with three or more unsaturated bonds, and more preferably 4 or more double bonds, and more preferably 5 or more double bonds, and even more preferably 6 or more double bonds, then this microbial strain is identified as a likely candidate to possess a novel PUFA PKS system of the type described in this invention. Screening this organism under the low oxygen conditions described above, and confirming production of bioactive molecules containing two or more unsaturated bonds would suggest the existence of a novel PUFA PKS system in the organism, which could be further confirmed by analysis of the microbes' genome.

The success of this method can also be enhanced by screening eukaryotic strains that are known to contain C17:0 and or C17:1 fatty acids (in conjunction with the large percentages of C14:0, C16:0 and C16:1 fatty acids described above)—because the C17:0 and C17:1 fatty acids are potential markers for a bacterial (prokaryotic) based or influenced fatty acid production system. Another marker for identifying strains containing novel PUFA PKS systems is the production of simple fatty acid profiles by the organism. According to the present invention, a "simple fatty acid profile" is defined as 8 or fewer fatty acids being produced by the strain at levels greater than 10% of total fatty acids.

Use of any of these methods or markers (singly or preferably in combination) would enable one of skill in the art to readily identify microbial strains that are highly predicted to contain a novel PUFA PKS system of the type described in this invention.

In a preferred embodiment combining many of the methods and markers described above, a novel biorational screen (using shake flask cultures) has been developed for detecting microorganisms containing PUFA producing PKS systems. This screening system is conducted as follows:

A portion of a culture of the strain/microorganism to be tested is placed in 250 mL baffled shake flask with 50 mL culture media (aerobic treatment), and another portion of culture of the same strain is placed in a 250 mL non-baffled shake flask with 200 mL culture medium (anoxic/low oxygen treatment). Various culture media can be employed depending on the type and strain of microorganism being evaluated. Both flasks are placed on a shaker table at 200 rpm. After 48-72 hr of culture time, the cultures are harvested by centrifugation and the cells are analyzed for fatty acid methyl ester content via gas chromatography to determine the following data for each culture: (1) fatty acid profile; (2) PUFA content; and (3) fat content (approximated as amount total fatty acids/cell dry weight).

These data are then analyzed asking the following five questions (Yes/No):

Comparing the Data from the Low O_2/Anoxic Flask with the Data from the Aerobic Flask:

(1) Did the DHA (or other PUFA content) (as % FAME (fatty acid methyl esters)) stay about the same or preferably increased in the low oxygen culture compared to the aerobic culture?

(2) Is C14:0+C16:0+C16:1 greater than about 40% TFA in the anoxic culture?

(3) Are there very little (<1% as FAME) or no precursors (C18:3n-3+C18:2n-6+C18:3n-6) to the conventional oxygen dependent elongase/desaturase pathway in the anoxic culture?

(4) Did fat content (as amount total fatty acids/cell dry weight) increase in the low oxygen culture compared to the aerobic culture?

(5) Did DHA (or other PUFA content) increase as % cell dry weight in the low oxygen culture compared to the aerobic culture?

If the first three questions are answered yes, this is a good indication that the strain contains a PKS genetic system for making long chain PUFAs. The more questions that are answered yes (preferably the first three questions must be answered yes), the stronger the indication that the strain contains such a PKS genetic system. If all five questions are answered yes, then there is a very strong indication that the strain contains a PKS genetic system for making long chain PUFAs. The lack of 18:3n-3/18:2n-6/18:3n-6 would indicate that the low oxygen conditions would have turned off or inhibited the conventional pathway for PUFA synthesis. A high 14:0/16:0/16:1 fatty is an preliminary indicator of a bacterially influenced fatty acid synthesis profile (the presence of C17:0 and 17:1 is also and indicator of this) and of a simple fatty acid profile. The increased PUFA synthesis and PUFA containing fat synthesis under the low oxygen conditions is directly indicative of a PUFA PKS system, since this system does not require oxygen to make highly unsaturated fatty acids.

Finally, in the identification method of the present invention, once a strong candidate is identified, the microbe is preferably screened to detect whether or not the microbe contains a PUFA PKS system. For example, the genome of the microbe can be screened to detect the presence of one or more nucleic acid sequences that encode a domain of a PUFA PKS system as described herein. Preferably, this step of detection includes a suitable nucleic acid detection method, such as hybridization, amplification and or sequencing of one or more nucleic acid sequences in the microbe of interest. The probes and/or primers used in the detection methods can be derived from any known PUFA PKS system, including the marine bacteria PUFA PKS systems described in U.S. Pat. No. 6,140,486, or the Thraustochytrid PUFA PKS systems described in U.S. application Ser. No. 09/231,899 and herein. Once novel PUFA PKS systems are identified, the genetic material from these systems can also be used to detect additional novel PUFA PKS systems. Methods of hybridization, amplification and sequencing of nucleic acids for the purpose of identification and detection of a sequence are well known in the art. Using these detection methods, sequence homology and domain structure (e.g., the presence, number and/or arrangement of various PUFA PKS functional domains) can be evaluated and compared to the known PUFA PKS systems described herein.

In some embodiments, a PUFA PKS system can be identified using biological assays. For example, in U.S. application Ser. No. 09/231,899, Example 7, the results of a key experiment using a well-known inhibitor of some types of fatty acid synthesis systems, i.e., thiolactomycin, is described. The inventors showed that the synthesis of PUFAs in whole cells of *Schizochytrium* could be specifically blocked without blocking the synthesis of short chain saturated fatty acids. The significance of this result is as follows: the inventors knew from analysis of cDNA sequences from *Schizochytrium* that a Type I fatty acid synthase system is present in *Schizochytrium*. It was known that thiolactomycin does not inhibit Type I FAS systems, and this is consistent with the inventors' data—i.e., production of the saturated fatty acids (primarily C14:0 and C16:0 in *Schizochytrium*) was not inhibited by the thiolactomycin treatment. There are no indications in the literature or in the inventors' own data that thiolactomycin has any inhibitory effect on the elongation of C14:0 or C16:0 fatty acids or their desaturation (i.e. the conversion of short chain saturated fatty acids to PUFAs by the classical pathway). Therefore, the fact that the PUFA production in *Schizochytrium* was blocked by thiolactomycin strongly indicates that the classical PUFA synthesis pathway does not produce the PUFAs in *Schizochytrium*, but rather that a different pathway of synthesis is involved. Further, it had previously been determined that the *Shewanella* PUFA PKS system is inhibited by thiolactomycin (note that the PUFA PKS system of the present invention has elements of both Type I and Type II systems), and it was known that thiolactomycin is an inhibitor of Type II FAS systems (such as that found in *E. coli*). Therefore, this experiment indicated that *Schizochytrium* produced PUFAs as a result of a pathway not involving the Type I FAS. A similar rationale and detection step could be used to detect a PUFA PKS system in a microbe identified using the novel screening method disclosed herein.

In addition, Example 3 shows additional biochemical data which provides evidence that PUFAs in *Schizochytrium* are not produced by the classical pathway (i.e., precursor product kinetics between C16:0 and DHA are not observed in whole cells and, in vitro PUFA synthesis can be separated from the membrane fraction—all of the fatty acid desaturases of the classical PUFA synthesis pathway, with the exception of the delta 9 desaturase which inserts the first double bond of the series, are associated with cellular membranes). This type of biochemical data could be used to detect PUFA PKS activity in microbe identified by the novel screening method described above.

Preferred microbial strains to screen using the screening/identification method of the present invention are chosen from the group consisting of: bacteria, algae, fungi, protozoa or protists, but most preferably from the eukaryotic microbes consisting of algae, fungi, protozoa and protists. These microbes are preferably capable of growth and production of the bioactive compounds containing two or more unsaturated bonds at temperatures greater than about 15° C., more preferably greater than about 20° C., even more preferably greater than about 25° C. and most preferably greater than about 30° C.

In some embodiments of this method of the present invention, novel bacterial PUFA PKS systems can be identified in bacteria that produce PUFAs at temperatures exceeding about 20° C., preferably exceeding about 25° C. and even more preferably exceeding about 30° C. As described previously herein, the marine bacteria, *Shewanella* and *Vibrio marinus*, described in U.S. Pat. No. 6,140,486, do not produce PUFAs at higher temperatures, which limits the usefulness of PUFA PKS systems derived from these bacteria, particularly in plant applications under field conditions. Therefore, in one

embodiment, the screening method of the present invention can be used to identify bacteria that have a PUFA PKS system which are capable of growth and PUFA production at higher temperatures (e.g., above about 20, 25, or 30° C.). In this embodiment, inhibitors of eukaryotic growth such as nystatin (antifungal) or cycloheximide (inhibitor of eukaryotic protein synthesis) can be added to agar plates used to culture/select initial strains from water samples/soil samples collected from the types of habitats/niches described below. This process would help select for enrichment of bacterial strains without (or minimal) contamination of eukaryotic strains. This selection process, in combination with culturing the plates at elevated temperatures (e.g. 30° C.), and then selecting strains that produce at least one PUFA would initially identify candidate bacterial strains with a PUFA PKS system that is operative at elevated temperatures (as opposed to those bacterial strains in the prior art which only exhibit PUFA production at temperatures less than about 20° C. and more preferably below about 5° C.).

Locations for collection of the preferred types of microbes for screening for a PUFA PKS system according to the present invention include any of the following: low oxygen environments (or locations near these types of low oxygen environments including in the guts of animals including invertebrates that consume microbes or microbe-containing foods (including types of filter feeding organisms), low or non-oxygen containing aquatic habitats (including freshwater, saline and marine), and especially at- or near-low oxygen environments (regions) in the oceans. The microbial strains would preferably not be obligate anaerobes but be adapted to live in both aerobic and low or anoxic environments. Soil environments containing both aerobic and low oxygen or anoxic environments would also excellent environments to find these organisms in and especially in these types of soil in aquatic habitats or temporary aquatic habitats.

A particularly preferred microbial strain would be a strain (selected from the group consisting of algae, fungi (including yeast), protozoa or protists) that, during a portion of its life cycle, is capable of consuming whole bacterial cells (bacterivory) by mechanisms such as phagocytosis, phagotrophic or endocytic capability and/or has a stage of its life cycle in which it exists as an amoeboid stage or naked protoplast. This method of nutrition would greatly increase the potential for transfer of a bacterial PKS system into a eukaryotic cell if a mistake occurred and the bacterial cell (or its DNA) did not get digested and instead are functionally incorporated into the eukaryotic cell.

Strains of microbes (other than the members of the Thraustochytrids) capable of bacterivory (especially by phagocytosis or endocytosis) can be found in the following microbial classes (including but not limited to example genera):

In the algae and algae-like microbes (including stramenopiles): of the class Euglenophyceae (for example genera *Euglena*, and *Peranema*), the class Chrysophyceae (for example the genus *Ochromonas*), the class Dinobryaceae (for example the genera *Dinobryon, Platychysis*, and *Chysochromulina*), the Dinophyceae (including the genera *Cryptheco-dinium, Gymnodinium, Peridinium, Ceratium, Gyrodinium*, and *Oxyrrhis*), the class Cryptophyceae (for example the genera *Cryptomonas*, and *Rhodomonas*), the class Xanthophyceae (for example the genus *Olisthodiscus*) (and including forms of algae in which an amoeboid stage occurs as in the flagellates Rhizochloridaceae, and zoospores/gametes of *Aphanochaete pascheri, Bumilleria stigeoclonium* and *Vaucheria geminata*), the class Eustigmatophyceae, and the class Prymnesiopyceae (including the genera *Prymnesium* and *Diacronema*).

In the Stramenopiles including the: Proteromonads, Opalines, Developayella, Diplophorys, Larbrinthulids, Thraustochytrids, Bicosecids, Oomycetes, Hypochytridiomycetes, *Commation, Reticulosphaera, Pelagomonas, Pelapococcus, Ollicola, Aureococcus*, Parmales, Raphidiophytes, Synurids, Rhizochromulinaales, Pedinellales, Dictyochales, Chrysomeridales, Sarcinochrysidales, Hydrurales, Hibberdiales, and Chromulinales.

In the Fungi: Class Myxomycetes (form myxamoebae)—slime molds, class Acrasieae including the orders Acrasiceae (for example the genus *Sappinia*), class Guttulinaceae (for example the genera *Guttulinopsis*, and *Guttulina*), class Dictysteliaceae (for example the genera *Acrasis, Dictyostelium, Polysphondylium*, and *Coenonia*), and class Phycomyceae including the orders Chytridiales, Ancylistales, Blastocladiales, Monoblepharidales, Saprolegniales, Peronosporales, Mucorales, and Entomophthorales.

In the Protozoa: Protozoa strains with life stages capable of bacterivory (including by phageocytosis) can be selected from the types classified as ciliates, flagellates or amoebae. Protozoan ciliates include the groups: Chonotrichs, Colpodids, Cyrtophores, Haptorids, Karyorelicts, Oligohymenophora, Polyhymenophora (spirotrichs), Prostomes and Suctoria. Protozoan flagellates include the Biosoecids, Bodonids, Cercomonads, Chrysophytes (for example the genera *Anthophysa, Chrysamoemba, Chrysosphaerella, Dendromonas, Dinobryon, Mallomonas, Ochromonas, Paraphysomonas, Poterioochromonas, Spumella, Syncrypta, Synura*, and *Uroglena*), Collar flagellates, Cryptophytes (for example the genera *Chilomonas, Cryptomonas, Cyanomonas*, and *Goniomonas*), Dinoflagellates, Diplomonads, Euglenoids, Heterolobosea, Pedinellids, Pelobionts, Phalansteriids, Pseudodendromonads, Spongomonads and Volvocales (and other flagellates including the unassigned flagellate genera of *Artodiscus, Clautriavia, Helkesimastix, Kathablepharis* and *Multicilia*). Amoeboid protozoans include the groups: Actinophryids, Centrohelids, Desmothoricids, Diplophryids, Eumamoebae, Heterolobosea, Leptomyxids, Nucleariid filose amoebae, Pelebionts, Testate amoebae and Vampyrellids (and including the unassigned amoebid genera *Gymnophrys, Biomyxa, Microcometes, Reticulomyxa, Belonocystis, Elaeorhanis, Allelogromia, Gromia* or *Lieberkuhnia*). The protozoan orders include the following: Percolomonadeae, Heterolobosea, Lyromonadea, Pseudociliata, Trichomonadea, Hypermastigea, Heteromiteae, Telonemea, Cyathobodonea, Ebridea, Pyytomyxea, Opalinea, Kinetomonadea, Hemimastigea, Protostelea, Myxagastrea, Dictyostelea, Choanomonadea, Apicomonadea, Eogregarinea, Neogregarinea, Coelotrolphea, Eucoccidea, Haemosporea, Piroplasmea, Spirotrichea, Prostomatea, Litostomatea, Phyllopharyngea, Nassophorea, Oligohymenophorea, Colpodea, Karyorelicta, Nucleohelea, Centrohelea, Acantharea, Sticholonchea, Polycystinea, Phaeodarea, Lobosea, Filosea, Athalamea, Monothalamea, Polythalamea, Xenophyophorea, Schizocladea, Holosea, Entamoebea, Myxosporea, Actinomyxea, Halosporea, Paramyxea, Rhombozoa and Orthonectea.

A preferred embodiment of the present invention includes strains of the microorganisms listed above that have been collected from one of the preferred habitats listed above.

One embodiment of the present invention relates to any microorganisms identified using the novel PUFA PKS screening method described above, to the PUFA PKS genes and proteins encoded thereby, and to the use of such microorganisms and/or PUFA PKS genes and proteins (including homologues and fragments thereof) in any of the methods described herein. In particular, the present invention encom-

passes organisms identified by the screening method of the present invention which are then genetically modified to regulate the production of bioactive molecules by said PUFA PKS system.

Yet another embodiment of the present invention relates to an isolated nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain or biologically active fragment thereof of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism. As discussed above, the present inventors have successfully used the method to identify a non-bacterial microorganism that has a PUFA PKS system to identify additional members of the order Thraustochytriales which contain a PUFA PKS system. The identification of three such microorganisms is described in Example 2. Specifically, the present inventors have used the screening method of the present invention to identify *Thraustochytrium* sp. 23B (ATCC 20892) as being highly predicted to contain a PUFA PKS system, followed by detection of sequences in the *Thraustochytrium* sp. 23B genome that hybridize to the *Schizochytrium* PUFAPKS genes disclosed herein. *Schizochytrium* limacium (IFO 32693) and *Ulkenia* (BP-5601) have also been identified as good candidates for containing PUFA PKS systems. Based on these data and on the similarities among members of the order Thraustochytriales, it is believed that many other Thraustochytriales PUFA PKS systems can now be readily identified using the methods and tools provided by the present invention. Therefore, Thraustochytriales PUFA PKS systems and portions and/or homologues thereof (e.g., proteins, domains and fragments thereof), genetically modified organisms comprising such systems and portions and/or homologues thereof, and methods of using such microorganisms and PUFA PKS systems, are encompassed by the present invention.

Developments have resulted in revision of the taxonomy of the Thraustochytrids. Taxonomic theorists place Thraustochytrids with the algae or algae-like protists. However, because of taxonomic uncertainty, it would be best for the purposes of the present invention to consider the strains described in the present invention as Thraustochytrids (Order: Thraustochytriales; Family: Thraustochytriaceae; Genus: *Thraustochytrium, Schizochytrium, Labyrinthuloides*, or *Japonochytrium*). For the present invention, members of the labrinthulids are considered to be included in the Thraustochytrids. Taxonomic changes are summarized below. Strains of certain unicellular microorganisms disclosed herein are members of the order Thraustochytriales. Thraustochytrids are marine eukaryotes with a evolving taxonomic history. Problems with the taxonomic placement of the Thraustochytrids have been reviewed by Moss (1986), Bahnweb and Jackle (1986) and Chamberlain and Moss (1988). According to the present invention, the phrases "Thraustochytrid", "Thraustochytriales microorganism" and "microorganism of the order Thraustochytriales" can be used interchangeably.

For convenience purposes, the Thraustochytrids were first placed by taxonomists with other colorless zoosporic eukaryotes in the Phycomycetes (algae-like fungi). The name Phycomycetes, however, was eventually dropped from taxonomic status, and the Thraustochytrids were retained in the Oomycetes (the biflagellate zoosporic fungi). It was initially assumed that the Oomycetes were related to the heterokont algae, and eventually a wide range of ultrastructural and biochemical studies, summarized by Barr (Barr, 1981, *Biosystems* 14:359-370) supported this assumption. The Oomycetes were in fact accepted by Leedale (Leedale, 1974, *Taxon* 23:261-270) and other phycologists as part of the heterokont algae. However, as a matter of convenience resulting from their heterotrophic nature, the Oomycetes and Thraustochytrids have been largely studied by mycologists (scientists who study fungi) rather than phycologists (scientists who study algae).

From another taxonomic perspective, evolutionary biologists have developed two general schools of thought as to how eukaryotes evolved. One theory proposes an exogenous origin of membrane-bound organelles through a series of endosymbioses (Margulis, 1970, *Origin of Eukaryotic Cells*. Yale University Press, New Haven); e.g., mitochondria were derived from bacterial endosymbionts, chloroplasts from cyanophytes, and flagella from spirochaetes. The other theory suggests a gradual evolution of the membrane-bound organelles from the non-membrane-bounded systems of the prokaryote ancestor via an autogenous process (Cavalier-Smith, 1975, *Nature* (Lond.) 256:462-468). Both groups of evolutionary biologists however, have removed the Oomycetes and Thraustochytrids from the fungi and place them either with the chromophyte algae in the kingdom Chromophyta (Cavalier-Smith, 1981, *BioSystems* 14:461-481) (this kingdom has been more recently expanded to include other protists and members of this kingdom are now called Stramenopiles) or with all algae in the kingdom Protoctista (Margulis and Sagen, 1985, *Biosystems* 18:141-147).

With the development of electron microscopy, studies on the ultrastructure of the zoospores of two genera of Thraustochytrids, *Thraustochytrium* and *Schizochytrium*, (Perkins, 1976, pp. 279-312 in "Recent Advances in Aquatic Mycology" (ed. E. B. G. Jones), John Wiley & Sons, New York; Kazama, 1980, *Can. J. Bot.* 58:2434-2446; Barr, 1981, *Biosystems* 14:359-370) have provided good evidence that the Thraustochytriaceae are only distantly related to the Oomycetes. Additionally, genetic data representing a correspondence analysis (a form of multivariate statistics) of 5 S ribosomal RNA sequences indicate that Thraustochytriales are clearly a unique group of eukaryotes, completely separate from the fungi, and most closely related to the red and brown algae, and to members of the Oomycetes (Mannella, et al., 1987, *Mol. Evol.* 24:228-235). Most taxonomists have agreed to remove the Thraustochytrids from the Oomycetes (Bartnicki-Garcia, 1987, pp. 389-403 in "Evolutionary Biology of the Fungi" (eds. Rayner, A. D. M., Brasier, C. M. & Moore, D.), Cambridge University Press, Cambridge).

In summary, employing the taxonomic system of Cavalier-Smith (Cavalier-Smith, 1981, *BioSystems* 14:461-481, 1983; Cavalier-Smith, 1993, *Microbiol Rev.* 57:953-994), the Thraustochytrids are classified with the chromophyte algae in the kingdom Chromophyta (Stramenopiles). This taxonomic placement has been more recently reaffirmed by Cavalier-Smith et al. using the 18s rRNA signatures of the Heterokonta to demonstrate that Thraustochytrids are chromists not Fungi (Cavalier-Smith et al., 1994, *Phil. Tran. Roy. Soc. London Series BioSciences* 346:387-397). This places them in a completely different kingdom from the fungi, which are all placed in the kingdom Eufungi. The taxonomic placement of the Thraustochytrids is therefore summarized below:

Kingdom: Chromophyta (Stramenopiles)
Phylum: Heterokonta
Order: Thraustochytriales
Family: Thraustochytriaceae
Genus: *Thraustochytrium, Schizochytrium, Labyrinthuloides*, or *Japonochytrium*

Some early taxonomists separated a few original members of the genus *Thraustochytrium* (those with an amoeboid life stage) into a separate genus called *Ulkenia*. However it is now known that most, if not all, Thraustochytrids (including

Thraustochytrium and *Schizochytrium*), exhibit amoeboid stages and as such, *Ulkenia* is not considered by some to be a valid genus. As used herein, the genus *Thraustochytrium* will include *Ulkenia.*

Despite the uncertainty of taxonomic placement within higher classifications of Phylum and Kingdom, the Thraustochytrids remain a distinctive and characteristic grouping whose members remain classifiable within the order Thraustochytriales.

Polyunsaturated fatty acids (PUFAs) are essential membrane components in higher eukaryotes and the precursors of many lipid-derived signaling molecules. The PUFA PKS system of the present invention uses pathways for PUFA synthesis that do not require desaturation and elongation of saturated fatty acids. The pathways catalyzed by PUFA PKS s that are distinct from previously recognized PKSs in both structure and mechanism. Generation of cis double bonds is suggested to involve position-specific isomerases; these enzymes are believed to be useful in the production of new families of antibiotics.

To produce significantly high yields of various bioactive molecules using the PUFA PKS system of the present invention, an organism, preferably a microorganism or a plant, can be genetically modified to affect the activity of a PUFA PKS system. In one aspect, such an organism can endogenously contain and express a PUFA PKS system, and the genetic modification can be a genetic modification of one or more of the functional domains of the endogenous PUFA PKS system, whereby the modification has some effect on the activity of the PUFA PKS system. In another aspect, such an organism can endogenously contain and express a PUFA PKS system, and the genetic modification can be an introduction of at least one exogenous nucleic acid sequence (e.g., a recombinant nucleic acid molecule), wherein the exogenous nucleic acid sequence encodes at least one biologically active domain or protein from a second PKS system and/or a protein that affects the activity of said PUFA PKS system (e.g., a phosphopantetheinyl transferases (PPTase), discussed below). In yet another aspect, the organism does not necessarily endogenously (naturally) contain a PUFA PKS system, but is genetically modified to introduce at least one recombinant nucleic acid molecule encoding an amino acid sequence having the biological activity of at least one domain of a PUFA PKS system. In this aspect, PUFA PKS activity is affected by introducing or increasing PUFA PKS activity in the organism. Various embodiments associated with each of these aspects will be discussed in greater detail below.

Therefore, according to the present invention, one embodiment relates to a genetically modified microorganism, wherein the microorganism expresses a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The at least one domain of the PUFA PKS system is encoded by a nucleic acid sequence chosen from: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS system from a microorganism identified by a screening method of the present invention; (c) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and, (d) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. The genetic modification affects the activity of the PKS system in the organism. The screening process referenced in part (b) has been described in detail above and includes the steps of: (a) selecting a microorganism that produces at least one PUFA; and, (b) identifying a microorganism from (a) that has an ability to produce increased PUFAs under dissolved oxygen conditions of less than about 5% of saturation in the fermentation medium, as compared to production of PUFAs by the microorganism under dissolved oxygen conditions of greater than about 5% of saturation, and preferably about 10%, and more preferably about 15%, and more preferably about 20% of saturation in the fermentation medium. The genetically modified microorganism can include any one or more of the above-identified nucleic acid sequences, and/or any of the other homologues of any of the *Schizochytrium* PUFA PKS ORFs or domains as described in detail above.

As used herein, a genetically modified microorganism can include a genetically modified bacterium, protist, microalgae, fungus, or other microbe, and particularly, any of the genera of the order Thraustochytriales (e.g., a Thraustochytrid) described herein (e.g., *Schizochytrium, Thraustochytrium, Japonochytrium, Labyrinthuloides*). Such a genetically modified microorganism has a genome which is modified (i.e., mutated or changed) from its normal (i.e., wild-type or naturally occurring) form such that the desired result is achieved (i.e., increased or modified PUFA PKS activity and/or production of a desired product using the PKS system). Genetic modification of a microorganism can be accomplished using classical strain development and/or molecular genetic techniques. Such techniques known in the art and are generally disclosed for microorganisms, for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press. The reference Sambrook et al., ibid., is incorporated by reference herein in its entirety. A genetically modified microorganism can include a microorganism in which nucleic acid molecules have been inserted, deleted or modified (i.e., mutated; e.g., by insertion, deletion, substitution, and/or inversion of nucleotides), in such a manner that such modifications provide the desired effect within the microorganism.

Preferred microorganism host cells to modify according to the present invention include, but are not limited to, any bacteria, protist, microalga, fungus, or protozoa. In one aspect, preferred microorganisms to genetically modify include, but are not limited to, any microorganism of the order Thraustochytriales. Particularly preferred host cells for use in the present invention could include microorganisms from a genus including, but not limited to: *Thraustochytrium, Labyrinthuloides, Japonochytrium*, and *Schizochytrium*. Preferred species within these genera include, but are not limited to: any *Schizochytrium* species, including *Schizochytrium* aggregatun, *Schizochytrium* limacinum, *Schizochytrium* minutum; any *Thraustochytrium* species (including former *Ulkenia* species such as *U. visurgensis, U. amoeboida, U. sarkariana, U. profunda, U. radiata, U. minuta* and *Ulkenia* sp. BP-5601), and including *Thraustochytrium* striatum, *Thraustochytrium* aureum, *Thraustochytrium* roseum; and any *Japonochytrium* species. Particularly preferred strains of Thraustochytriales include, but are not limited to: *Schizochytrium* sp. (S31) (ATCC 20888); *Schizochytrium* sp. (S8)(ATCC 20889); *Schizochytrium* sp. (LC-RM)(ATCC 18915); *Schizochytrium*

sp. (SR21); *Schizochytrium* aggregatum (Goldstein et Belsky)(ATCC 28209); *Schizochytrium* limacinum (Honda et Yokochi)(IFO 32693); *Thraustochytrium* sp. (23B)(ATCC 20891); *Thraustochytrium* striatum (Schneider)(ATCC 24473); *Thraustochytrium aureum* (Goldstein)(ATCC 34304); *Thraustochytrium roseum* (Goldstein)(ATCC 28210); and *Japonochytrium* sp. (L1)(ATCC 28207). Other examples of suitable host microorganisms for genetic modification include, but are not limited to, yeast including *Saccharomyces cerevisiae, Saccharomyces carlsbergensis*, or other yeast such as *Candida, Kluyveromyces*, or other fungi, for example, filamentous fungi such as *Aspergillus, Neurospora, Penicillium*, etc. Bacterial cells also may be used as hosts. This includes *Escherichia coli*, which can be useful in fermentation processes. Alternatively, a host such as a *Lactobacillus* species or *Bacillus* species can be used as a host.

Another embodiment of the present invention relates to a genetically modified plant, wherein the plant has been genetically modified to recombinantly express a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. The domain is encoded by a nucleic acid sequence chosen from: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS system from a microorganism identified by the screening and selection method described herein (see brief summary of method in discussion of genetically modified microorganism above); (c) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and biologically active fragments thereof; (d) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (e) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and/or (f) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. The genetically modified plant can include any one or more of the above-identified nucleic acid sequences, and/or any of the other homologues of any of the *Schizochytrium* PUFA PKS ORFs or domains as described in detail above.

As used herein, a genetically modified plant can include any genetically modified plant including higher plants and particularly, any consumable plants or plants useful for producing a desired bioactive molecule of the present invention. Such a genetically modified plant has a genome which is modified (i.e., mutated or changed) from its normal (i.e., wild-type or naturally occurring) form such that the desired result is achieved (i.e., increased or modified PUFA PKS activity and/or production of a desired product using the PKS system). Genetic modification of a plant can be accomplished using classical strain development and/or molecular genetic techniques. Methods for producing a transgenic plant, wherein a recombinant nucleic acid molecule encoding a desired amino acid sequence is incorporated into the genome of the plant, are known in the art. A preferred plant to genetically modify according to the present invention is preferably a plant suitable for consumption by animals, including humans.

Preferred plants to genetically modify according to the present invention (i.e., plant host cells) include, but are not limited to any higher plants, and particularly consumable plants, including crop plants and especially plants used for their oils. Such plants can include, for example: canola, soybeans, rapeseed, linseed, corn, safflowers, sunflowers and tobacco. Other preferred plants include those plants that are known to produce compounds used as pharmaceutical agents, flavoring agents, neutraceutical agents, functional food ingredients or cosmetically active agents or plants that are genetically engineered to produce these compounds/agents.

According to the present invention, a genetically modified microorganism or plant includes a microorganism or plant that has been modified using recombinant technology. As used herein, genetic modifications which result in a decrease in gene expression, in the function of the gene, or in the function of the gene product (i.e., the protein encoded by the gene) can be referred to as inactivation (complete or partial), deletion, interruption, blockage or down-regulation of a gene. For example, a genetic modification in a gene which results in a decrease in the function of the protein encoded by such gene, can be the result of a complete deletion of the gene (i.e., the gene does not exist, and therefore the protein does not exist), a mutation in the gene which results in incomplete or no translation of the protein (e.g., the protein is not expressed), or a mutation in the gene which decreases or abolishes the natural function of the protein (e.g., a protein is expressed which has decreased or no enzymatic activity or action). Genetic modifications that result in an increase in gene expression or function can be referred to as amplification, overproduction, overexpression, activation, enhancement, addition, or up-regulation of a gene.

The genetic modification of a microorganism or plant according to the present invention preferably affects the activity of the PKS system expressed by the plant, whether the PKS system is endogenous and genetically modified, endogenous with the introduction of recombinant nucleic acid molecules into the organism, or provided completely by recombinant technology. According to the present invention, to "affect the activity of a PKS system" includes any genetic modification that causes any detectable or measurable change or modification in the PKS system expressed by the organism as compared to in the absence of the genetic modification. A detectable change or modification in the PKS system can include, but is not limited to: the introduction of PKS system activity into an organism such that the organism now has measurable/detectable PKS system activity (i.e., the organism did not contain a PKS system prior to the genetic modification), the introduction into the organism of a functional domain from a different PKS system than a PKS system endogenously expressed by the organism such that the PKS system activity is modified (e.g., a bacterial PUFA PKS domain or a type I PKS domain is introduced into an organism that endogenously expresses a non-bacterial PUFA PKS system), a change in the amount of a bioactive molecule produced by the PKS system (e.g., the system produces more (increased amount) or less (decreased amount) of a given product as compared to in the absence of the genetic modification), a change in the type of a bioactive molecule produced by the PKS system (e.g., the system produces a new or dif-

ferent product, or a variant of a product that is naturally produced by the system), and/or a change in the ratio of multiple bioactive molecules produced by the PKS system (e.g., the system produces a different ratio of one PUFA to another PUFA, produces a completely different lipid profile as compared to in the absence of the genetic modification, or places various PUFAs in different positions in a triacylglycerol as compared to the natural configuration). Such a genetic modification includes any type of genetic modification and specifically includes modifications made by recombinant technology and by classical mutagenesis.

It should be noted that reference to increasing the activity of a functional domain or protein in a PUFA PKS system refers to any genetic modification in the organism containing the domain or protein (or into which the domain or protein is to be introduced) which results in increased functionality of the domain or protein system and can include higher activity of the domain or protein (e.g., specific activity or in vivo enzymatic activity), reduced inhibition or degradation of the domain or protein system, and overexpression of the domain or protein. For example, gene copy number can be increased, expression levels can be increased by use of a promoter that gives higher levels of expression than that of the native promoter, or a gene can be altered by genetic engineering or classical mutagenesis to increase the activity of the domain or protein encoded by the gene.

Similarly, reference to decreasing the activity of a functional domain or protein in a PUFA PKS system refers to any genetic modification in the organism containing such domain or protein (or into which the domain or protein is to be introduced) which results in decreased functionality of the domain or protein and includes decreased activity of the domain or protein, increased inhibition or degradation of the domain or protein and a reduction or elimination of expression of the domain or protein. For example, the action of domain or protein of the present invention can be decreased by blocking or reducing the production of the domain or protein, "knocking out" the gene or portion thereof encoding the domain or protein, reducing domain or protein activity, or inhibiting the activity of the domain or protein. Blocking or reducing the production of an domain or protein can include placing the gene encoding the domain or protein under the control of a promoter that requires the presence of an inducing compound in the growth medium. By establishing conditions such that the inducer becomes depleted from the medium, the expression of the gene encoding the domain or protein (and therefore, of protein synthesis) could be turned off. Blocking or reducing the activity of domain or protein could also include using an excision technology approach similar to that described in U.S. Pat. No. 4,743,546, incorporated herein by reference. To use this approach, the gene encoding the protein of interest is cloned between specific genetic sequences that allow specific, controlled excision of the gene from the genome. Excision could be prompted by, for example, a shift in the cultivation temperature of the culture, as in U.S. Pat. No. 4,743,546, or by some other physical or nutritional signal.

In one embodiment of the present invention, a genetic modification includes a modification of a nucleic acid sequence encoding an amino acid sequence that has a biological activity of at least one domain of a non-bacterial PUFA PKS system as described herein. Such a modification can be to an amino acid sequence within an endogenously (naturally) expressed non-bacterial PUFA PKS system, whereby a microorganism that naturally contains such a system is genetically modified by, for example, classical mutagenesis and selection techniques and/or molecular genetic techniques, include genetic engineering techniques. Genetic engineering techniques can include, for example, using a targeting recombinant vector to delete a portion of an endogenous gene, or to replace a portion of an endogenous gene with a heterologous sequence. Examples of heterologous sequences that could be introduced into a host genome include sequences encoding at least one functional domain from another PKS system, such as a different non-bacterial PUFA PKS system, a bacterial PUFA PKS system, a type I PKS system, a type II PKS system, or a modular PKS system. Other heterologous sequences to introduce into the genome of a host includes a sequence encoding a protein or functional domain that is not a domain of a PKS system, but which will affect the activity of the endogenous PKS system. For example, one could introduce into the host genome a nucleic acid molecule encoding a phosphopantetheinyl transferase (discussed below). Specific modifications that could be made to an endogenous PUFA PKS system are discussed in detail below.

In another aspect of this embodiment of the invention, the genetic modification can include: (1) the introduction of a recombinant nucleic acid molecule encoding an amino acid sequence having a biological activity of at least one domain of a non-bacterial PUFA PKS system; and/or (2) the introduction of a recombinant nucleic acid molecule encoding a protein or functional domain that affects the activity of a PUFA PKS system, into a host. The host can include: (1) a host cell that does not express any PKS system, wherein all functional domains of a PKS system are introduced into the host cell, and wherein at least one functional domain is from a non-bacterial PUFA PKS system; (2) a host cell that expresses a PKS system (endogenous or recombinant) having at least one functional domain of a non-bacterial PUFA PKS system, wherein the introduced recombinant nucleic acid molecule can encode at least one additional non-bacterial PUFA PKS domain function or another protein or domain that affects the activity of the host PKS system; and (3) a host cell that expresses a PKS system (endogenous or recombinant) which does not necessarily include a domain function from a non-bacterial PUFA PKS, and wherein the introduced recombinant nucleic acid molecule includes a nucleic acid sequence encoding at least one functional domain of a non-bacterial PUFA PKS system. In other words, the present invention intends to encompass any genetically modified organism (e.g., microorganism or plant), wherein the organism comprises at least one non-bacterial PUFA PKS domain function (either endogenously or by recombinant modification), and wherein the genetic modification has a measurable effect on the non-bacterial PUFA PKS domain function or on the PKS system when the organism comprises a functional PKS system.

Therefore, using the non-bacterial PUFA PKS systems of the present invention, which, for example, makes use of genes from Thraustochytrid PUFA PKS systems, gene mixing can be used to extend the range of PUFA products to include EPA, DHA, ARA, GLA, SDA and others, as well as to produce a wide variety of bioactive molecules, including antibiotics, other pharmaceutical compounds, and other desirable products. The method to obtain these bioactive molecules includes not only the mixing of genes from various organisms but also various methods of genetically modifying the non-bacterial PUFA PKS genes disclosed herein. Knowledge of the genetic basis and domain structure of the non-bacterial PUFA PKS system of the present invention provides a basis for designing novel genetically modified organisms which produce a variety of bioactive molecules. Although mixing and modification of any PKS domains and related genes are contemplated

by the present inventors, by way of example, various possible manipulations of the PUFA-PKS system are discussed below with regard to genetic modification and bioactive molecule production.

For example, in one embodiment, non-bacterial PUFA-PKS system products, such as those produced by Thraustochytrids, are altered by modifying the CLF (chain length factor) domain. This domain is characteristic of Type II (dissociated enzymes) PKS systems. Its amino acid sequence shows homology to KS (keto synthase pairs) domains, but it lacks the active site cysteine. CLF may function to determine the number of elongation cycles, and hence the chain length, of the end product. In this embodiment of the invention, using the current state of knowledge of FAS and PKS synthesis, a rational strategy for production of ARA by directed modification of the non-bacterial PUFA-PKS system is provided. There is controversy in the literature concerning the function of the CLF in PKS systems (C. Bisang et al., *Nature* 401, 502 (1999)) and it is realized that other domains may be involved in determination of the chain length of the end product. However, it is significant that *Schizochytrium* produces both DHA (C22:6, ω-3) and DPA (C22:5, ω-6). In the PUFA-PKS system the cis double bonds are introduced during synthesis of the growing carbon chain. Since placement of the ω-3 and ω-6 double bonds occurs early in the synthesis of the molecules, one would not expect that they would affect subsequent end-product chain length determination. Thus, without being bound by theory, the present inventors believe that introduction of a factor (e.g. CLF) that directs synthesis of C20 units (instead of C22 units) into the *Schizochytrium* PUFA-PKS system will result in the production of EPA (C20:5, ω-3) and ARA (C20:4, ω-6). For example, in heterologous systems, one could exploit the CLF by directly substituting a CLF from an EPA producing system (such as one from *Photobacterium*) into the *Schizochytrium* gene set. The fatty acids of the resulting transformants can then be analyzed for alterations in profiles to identify the transformants producing EPA and/or ARA.

In addition to dependence on development of a heterologous system (recombinant system, such as could be introduced into plants), the CLF concept can be exploited in *Schizochytrium* (i.e., by modification of a *Schizochytrium* genome). Transformation and homologous recombination has been demonstrated in *Schizochytrium*. One can exploit this by constructing a clone with the CLF of OrfB replaced with a CLF from a C20 PUFA-PKS system. A marker gene will be inserted downstream of the coding region. One can then transform the wild type cells, select for the marker phenotype and then screen for those that had incorporated the new CLF. Again, one would analyze these for any effects on fatty acid profiles to identify transformants producing EPA and/or ARA. If some factor other than those associated with the CLF are found to influence the chain length of the end product, a similar strategy could be employed to alter those factors.

Another preferred embodiment involving alteration of the PUFA-PKS products involves modification or substitution of the β-hydroxy acyl-ACP dehydrase/keto synthase pairs. During cis-vaccenic acid (C18:1, Δ11) synthesis in *E. coli*, creation of the cis double bond is believed to depend on a specific DH enzyme, β-hydroxy acyl-ACP dehydrase, the product of the FabA gene. This enzyme removes HOH from a β-keto acyl-ACP and leaves a trans double bond in the carbon chain. A subset of DH's, FabA-like, possess cis-trans isomerase activity (Heath et al., 1996, supra). A novel aspect of bacterial and non-bacterial PUFA-PKS systems is the presence of two FabA-like DH domains. Without being bound by theory, the present inventors believe that one or both of these DH domains will possess cis-trans isomerase activity (manipulation of the DH domains is discussed in greater detail below).

Another aspect of the unsaturated fatty acid synthesis in *E. coli* is the requirement for a particular KS enzyme, β-ketoacyl-ACP synthase, the product of the FabB gene. This is the enzyme that carries out condensation of a fatty acid, linked to a cysteine residue at the active site (by a thio-ester bond), with a malonyl-ACP. In the multi-step reaction, CO_2 is released and the linear chain is extended by two carbons. It is believed that only this KS can extend a carbon chain that contains a double bond. This extension occurs only when the double bond is in the cis configuration; if it is in the trans configuration, the double bond is reduced by enoyl-ACP reductase (ER) prior to elongation (Heath et al., 1996, supra). All of the PUFA-PKS systems characterized so far have two KS domains, one of which shows greater homology to the FabB-like KS of *E. coli* than the other. Again, without being bound by theory, the present inventors believe that in PUFA-PKS systems, the specificities and interactions of the DH (FabA-like) and KS (FabB-like) enzymatic domains determine the number and placement of cis double bonds in the end products. Because the number of 2-carbon elongation reactions is greater than the number of double bonds present in the PUFA-PKS end products, it can be determined that in some extension cycles complete reduction occurs. Thus the DH and KS domains can be used as targets for alteration of the DHA/DPA ratio or ratios of other long chain fatty acids. These can be modified and/or evaluated by introduction of homologous domains from other systems or by mutagenesis of these gene fragments.

In another embodiment, the ER (enoyl-ACP reductase—an enzyme which reduces the trans-double bond in the fatty acyl-ACP resulting in fully saturated carbons) domains can be modified or substituted to change the type of product made by the PKS system. For example, the present inventors know that *Schizochytrium* PUFA-PKS system differs from the previously described bacterial systems in that it has two (rather than one) ER domains.

Without being bound by theory, the present inventors believe these ER domains can strongly influence the resulting PKS production product. The resulting PKS product could be changed by separately knocking out the individual domains or by modifying their nucleotide sequence or by substitution of ER domains from other organisms.

In another embodiment, nucleic acid molecules encoding proteins or domains that are not part of a PKS system, but which affect a PKS system, can be introduced into an organism. For example, all of the PUFA PKS systems described above contain multiple, tandem, ACP domains. ACP (as a separate protein or as a domain of a larger protein) requires attachment of a phosphopantetheine cofactor to produce the active, holo-ACP. Attachment of phosphopantetheine to the apo-ACP is carried out by members of the superfamily of enzymes—the phosphopantetheinyl transferases (PPTase) (Lambalot R. H., et al., *Chemistry and Biology,* 3, 923 (1996)).

By analogy to other PKS and FAS systems, the present inventors presume that activation of the multiple ACP domains present in the *Schizochytrium* ORFA protein is carried out by a specific, endogenous, PPTase. The gene encoding this presumed PPTase has not yet been identified in *Schizochytrium*. If such a gene is present in *Schizochytrium*, one can envision several approaches that could be used in an attempt to identify and clone it. These could include (but would not be limited to): generation and partial sequencing of a cDNA library prepared from actively growing

Schizochytrium cells (note, one sequence was identified in the currently available *Schizochytrium* cDNA library set which showed homology to PPTase's; however, it appears to be part of a multidomain FAS protein, and as such may not encode the desired OrfA specific PPTase); use of degenerate oligonucleotide primers designed using amino acid motifs present in many PPTase's in PCR reactions (to obtain a nucleic acid probe molecule to screen genomic or cDNA libraries); genetic approaches based on protein-protein interactions (e.g. a yeast two-hybrid system) in which the ORFA-ACP domains would be used as a "bait" to find a "target" (i.e. the PPTase); and purification and partial sequencing of the enzyme itself as a means to generate a nucleic acid probe for screening of genomic or cDNA libraries.

It is also conceivable that a heterologous PPTase may be capable of activating the *Schizochytrium* ORFA ACP domains. It has been shown that some PPTases, for example the sfp enzyme of *Bacillus subtilis* (Lambalot et al., supra) and the svp enzyme of *Streptomyces* verticillus (Sanchez et al., 2001, *Chemistry & Biology* 8:725-738), have a broad substrate tolerance. These enzymes can be tested to see if they will activate the *Schizochytrium* ACP domains. Also, a recent publication described the expression of a fungal PKS protein in tobacco (Yalpani et al., 2001, *The Plant Cell* 13:1401-1409). Products of the introduced PKS system (encoded by the 6-methylsalicyclic acid synthase gene of *Penicillium patulum*) were detected in the transgenic plant, even though the corresponding fungal PPTase was not present in those plants. This suggested that an endogenous plant PPTase(s) recognized and activated the fungal PKS ACP domain. Of relevance to this observation, the present inventors have identified two sequences (genes) in the *Arabidopsis* whole genome database that are likely to encode PPTases. These sequences (GenBank Accession numbers; AAG51443 and AAC05345) are currently listed as encoding "Unknown Proteins". They can be identified as putative PPTases based on the presence in the translated protein sequences of several signature motifs including; G(I/V)D and WxxKE(A/S)xxK (SEQ ID NO:33), (listed in Lambalot et al., 1996 as characteristic of all PPTases). In addition, these two putative proteins contain two additional motifs typically found in PPTases typically associated with PKS and non-ribosomal peptide synthesis systems; i.e., FN(I/L/V)SHS (SEQ ID NO:34) and (I/V/L)G(I/L/V)D(I/L/V) (SEQ ID NO:35). Furthermore, these motifs occur in the expected relative positions in the protein sequences. It is likely that homologues of the *Arabidopsis* genes are present in other plants, such as tobacco. Again, these genes can be cloned and expressed to see if the enzymes they encode can activate the *Schizochytrium* ORFA ACP domains, or alternatively, OrfA could be expressed directly in the transgenic plant (either targeted to the plastid or the cytoplasm).

Another heterologous PPTase which may recognize the ORFA ACP domains as substrates is the Het I protein of *Nostoc* sp. PCC 7120 (formerly called *Anabaena* sp. PCC 7120). As noted in U.S. Pat. No. 6,140,486, several of the PUFA-PKS genes of *Shewanella* showed a high degree of homology to protein domains present in a PKS cluster found in *Nostoc* (FIG. **2** of that patent). This *Nostoc* PKS system is associated with the synthesis of long chain (C26 or C28) hydroxy fatty acids that become esterified to sugar moieties and form a part of the heterocyst cell wall. These *Nostoc* PKS domains are also highly homologous to the domains found in Orfs B and C of the *Schizochytrium* PKS proteins (i.e. the same ones that correspond to those found in the *Shewanella* PKS proteins). Until very recently, none of the *Nostoc* PKS domains present in the GenBank databases showed high homology to any of the domains of *Schizochytrium* OrfA (or the homologous *Shewanella* Orf 5 protein). However, the complete genome of *Nostoc* has recently been sequenced and as a result, the sequence of the region just upstream of the PKS gene cluster is now available. In this region are three Orfs that show homology to the domains (KS, MAT, ACP and KR) of OrfA (see FIG. **3**). Included in this set are two ACP domains, both of which show high homology to the ORFA ACP domains. At the end of the *Nostoc* PKS cluster is the gene that encodes the Het I PPTase. Previously, it was not obvious what the substrate of the Het I enzyme could be, however the presence of tandem ACP domains in the newly identified Orf (Hgl E) of the cluster strongly suggests to the present inventors that it is those ACPs. The homology of the ACP domains of *Schizochytrium* and *Nostoc*, as well as the tandem arrangement of the domains in both proteins, makes Het I a likely candidate for heterologous activation of the *Schizochytrium* ORFA ACPs. The present inventors are believed to be the first to recognize and contemplate this use for *Nostoc* Het I PPTase.

As indicated in Metz et al., 2001, supra, one novel feature of the PUFA PKS systems is the presence of two dehydratase domains, both of which show homology to the FabA proteins of *E. coli*. With the availability of the new *Nostoc* PKS gene sequences mentioned above, one can now compare the two systems and their products. The sequence of domains in the *Nostoc* cluster (from HglE to Het I) as the present inventors have defined them is (see FIG. **3**):

KS-MAT-2×ACP, KR, KS, CLF-AT, ER (HetM, HetN) HetI

In the *Schizochytrium* PUFA-PKS Orfs A, B & C the sequence (OrfA-B-C) is:

KS-MAT-9xACP-KR KS-CLF-AT-ER DH-DH-ER

One can see the correspondence of the domains sequence (there is also a high amino acid sequence homology). The product of the *Nostoc* PKS system is a long chain hydroxy fatty acid (C26 or C28 with one or two hydroxy groups) that contains no double bonds (cis or trans). The product of the *Schizochytrium* PKS system is a long chain polyunsaturated fatty acid (C22, with 5 or 6 double bonds—all cis). An obvious difference between the two domain sets is the presence of the two DH domains in the *Schizochytrium* proteins—just the domains implicated in the formation of the cis double bonds of DHA and DPA (presumably HetM and HetN in the *Nostoc* system are involved in inclusion of the hydroxyl groups and also contain a DH domain whose origin differs from the those found in the PUFA). Also, the role of the duplicated ER domain in the *Schizochytrium* Orfs B and C is not known (the second ER domain in is not present other characterized PUFA PKS systems). The amino acid sequence homology between the two sets of domains implies an evolutionary relationship. One can conceive of the PUFA PKS gene set being derived from (in an evolutionary sense) an ancestral *Nostoc*-like PKS gene set by incorporation of the DH (FabA-like) domains. The addition of the DH domains would result in the introduction of cis double bonds in the new PKS end product structure.

The comparisons of the *Schizochytrium* and *Nostoc* PKS domain structures as well as the comparison of the domain organization between the *Schizochytrium* and *Shewanella* PUFA-PKS proteins demonstrate nature's ability to alter domain order as well as incorporate new domains to create novel end products. In addition, the genes can now be manipulated in the laboratory to create new products. The implication from these observations is that it should be possible to continue to manipulate the systems in either a directed or random way to influence the end products. For example, in

a preferred embodiment, one could envision substituting one of the DH (FabA-like) domains of the PUFA-PKS system for a DH domain that did not posses isomerization activity, potentially creating a molecule with a mix of cis- and trans-double bonds. The current products of the *Schizochytrium* PUFA PKS system are DHA and DPA (C22:5 ω6). If one manipulated the system to produce C20 fatty acids, one would expect the products to be EPA and ARA (C20:4 ω6). This could provide a new source for ARA. One could also substitute domains from related PUFA-PKS systems that produced a different DHA to DPA ratio—for example by using genes from *Thraustochytrium* 23B (the PUFA PKS system of which is identified for the first time herein).

Additionally, one could envision specifically altering one of the ER domains (e.g. removing, or inactivating) in the *Schizochytrium* PUFA PKS system (other PUFA PKS systems described so far do not have two ER domains) to determine its effect on the end product profile. Similar strategies could be attempted in a directed manner for each of the distinct domains of the PUFA-PKS proteins using more or less sophisticated approaches. Of course one would not be limited to the manipulation of single domains. Finally, one could extend the approach by mixing domains from the PUFA-PKS system and other PKS or FAS systems (e.g., type I, type II, modular) to create an entire range of new end products. For example, one could introduce the PUFA-PKS DH domains into systems that do not normally incorporate cis double bonds into their end products.

Accordingly, encompassed by the present invention are methods to genetically modify microbial or plant cells by: genetically modifying at least one nucleic acid sequence in the organism that encodes an amino acid sequence having the biological activity of at least one functional domain of a non-bacterial PUFA PKS system according to the present invention, and/or expressing at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding such amino acid sequence. Various embodiments of such sequences, methods to genetically modify an organism, and specific modifications have been described in detail above. Typically, the method is used to produce a particular genetically modified organism that produces a particular bioactive molecule or molecules.

One embodiment of the present invention relates to a recombinant host cell which has been modified to express a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the PKS catalyzes both iterative and non-iterative enzymatic reactions, and wherein the PUFA PKS system comprises: (a) at least two enoyl ACP-reductase (ER) domains; (b) at least six acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. In one embodiment, the PUFA PKS system is a eukaryotic PUFA PKS system. In a preferred embodiment, the PUFA PKS system is an algal PUFA PKS system. In a more preferred embodiment, the PUFA PKS system is a Thraustochytriales PUFA PKS system. Such PUFA PKS systems can include, but are not limited to, a *Schizochytrium* PUFA PKS system, and a *Thraustochytrium* PUFA PKS system. In one embodiment, the PUFA PKS system can be expressed in a prokaryotic host cell. In another embodiment, the PUFA PKS system can be expressed in a eukaryotic host cell.

Another embodiment of the present invention relates to a recombinant host cell which has been modified to express a non-bacterial PUFA PKS system, wherein the PKS system catalyzes both iterative and non-iterative enzymatic reactions, and wherein the non-bacterial PUFA PKS system comprises at least the following biologically active domains: (a) at least one enoyl ACP-reductase (ER) domain; (b) multiple acyl carrier protein (ACP) domains (at least four); (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. One aspect of this embodiment of the invention relates to a method to produce a product containing at least one PUFA, comprising growing a plant comprising any of the recombinant host cells described above, wherein the recombinant host cell is a plant cell, under conditions effective to produce the product. Another aspect of this embodiment of the invention relates to a method to produce a product containing at least one PUFA, comprising culturing a culture containing any of the recombinant host cells described above, wherein the host cell is a microbial cell, under conditions effective to produce the product. In a preferred embodiment, the PKS system in the host cell catalyzes the direct production of triglycerides.

Another embodiment of the present invention relates to a microorganism comprising a non-bacterial, polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the PKS catalyzes both iterative and non-iterative enzymatic reactions, and wherein the PUFA PKS system comprises: (a) at least two enoyl ACP-reductase (ER) domains; (b) at least six acyl carrier protein (ACP) domains; (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain. Preferably, the microorganism is a non-bacterial microorganism and more preferably, a eukaryotic microorganism.

Yet another embodiment of the present invention relates to a microorganism comprising a non-bacterial, polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system, wherein the PKS catalyzes both iterative and non-iterative enzymatic reactions, and wherein the PUFA PKS system comprises: (a) at least one enoyl ACP-reductase (ER) domain; (b) multiple acyl carrier protein (ACP) domains (at least four); (c) at least two β-keto acyl-ACP synthase (KS) domains; (d) at least one acyltransferase (AT) domain; (e) at least one ketoreductase (KR) domain; (f) at least two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains; (g) at least one chain length factor (CLF) domain; and (h) at least one malonyl-CoA:ACP acyltransferase (MAT) domain.

In one embodiment of the present invention, it is contemplated that a mutagenesis program could be combined with a selective screening process to obtain bioactive molecules of interest. This would include methods to search for a range of bioactive compounds. This search would not be restricted to production of those molecules with cis double bonds. The mutagenesis methods could include, but are not limited to: chemical mutagenesis, gene shuffling, switching regions of the genes encoding specific enzymatic domains, or mutagenesis restricted to specific regions of those genes, as well as other methods.

For example, high throughput mutagenesis methods could be used to influence or optimize production of the desired bioactive molecule. Once an effective model system has been developed, one could modify these genes in a high throughput

manner. Utilization of these technologies can be envisioned on two levels. First, if a sufficiently selective screen for production of a product of interest (e.g., ARA) can be devised, it could be used to attempt to alter the system to produce this product (e.g., in lieu of, or in concert with, other strategies such as those discussed above). Additionally, if the strategies outlined above resulted in a set of genes that did produce the product of interest, the high throughput technologies could then be used to optimize the system. For example, if the introduced domain only functioned at relatively low temperatures, selection methods could be devised to permit removing that limitation. In one embodiment of the invention, screening methods are used to identify additional non-bacterial organisms having novel PKS systems similar to the PUFA PKS system of *Schizochytrium*, as described herein (see above). Homologous PKS systems identified in such organisms can be used in methods similar to those described herein for the *Schizochytrium*, as well as for an additional source of genetic material from which to create, further modify and/or mutate a PKS system for expression in that microorganism, in another microorganism, or in a higher plant, to produce a variety of compounds.

It is recognized that many genetic alterations, either random or directed, which one may introduce into a native (endogenous, natural) PKS system, will result in an inactivation of enzymatic functions. A preferred embodiment of the invention includes a system to select for only those modifications that do not block the ability of the PKS system to produce a product. For example, the FabB—strain of *E. coli* is incapable of synthesizing unsaturated fatty acids and requires supplementation of the medium with fatty acids that can substitute for its normal unsaturated fatty acids in order to grow (see Metz et al., 2001, supra). However, this requirement (for supplementation of the medium) can be removed when the strain is transformed with a functional PUFA-PKS system (i.e. one that produces a PUFA product in the *E. coli* host—see (Metz et al., 2001, supra, FIG. **2**A). The transformed FabB—strain now requires a functional PUFA-PKS system (to produce the unsaturated fatty acids) for growth without supplementation. The key element in this example is that production of a wide range of unsaturated fatty acid will suffice (even unsaturated fatty acid substitutes such as branched chain fatty acids). Therefore, in another preferred embodiment of the invention, one could create a large number of mutations in one or more of the PUFA PKS genes disclosed herein, and then transform the appropriately modified FabB—strain (e.g. create mutations in an expression construct containing an ER domain and transform a FabB—strain having the other essential domains on a separate plasmid—or integrated into the chromosome) and select only for those transformants that grow without supplementation of the medium (i.e., that still possessed an ability to produce a molecule that could complement the FabB—defect). Additional screens could be developed to look for particular compounds (e.g. use of GC for fatty acids) being produced in this selective subset of an active PKS system. One could envision a number of similar selective screens for bioactive molecules of interest.

As described above, in one embodiment of the present invention, a genetically modified microorganism or plant includes a microorganism or plant which has an enhanced ability to synthesize desired bioactive molecules (products) or which has a newly introduced ability to synthesize specific products (e.g., to synthesize a specific antibiotic). According to the present invention, "an enhanced ability to synthesize" a product refers to any enhancement, or up-regulation, in a pathway related to the synthesis of the product such that the microorganism or plant produces an increased amount of the product (including any production of a product where there was none before) as compared to the wild-type microorganism or plant, cultured or grown, under the same conditions. Methods to produce such genetically modified organisms have been described in detail above.

One embodiment of the present invention is a method to produce desired bioactive molecules (also referred to as products or compounds) by growing or culturing a genetically modified microorganism or plant of the present invention (described in detail above). Such a method includes the step of culturing in a fermentation medium or growing in a suitable environment, such as soil, a microorganism or plant, respectively, that has a genetic modification as described previously herein and in accordance with the present invention. In a preferred embodiment, method to produce bioactive molecules of the present invention includes the step of culturing under conditions effective to produce the bioactive molecule a genetically modified organism that expresses a PKS system comprising at least one biologically active domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. In this preferred aspect, at least one domain of the PUFA PKS system is encoded by a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS system from a microorganism identified by the novel screening method of the present invention (described above in detail); (c) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and biologically active fragments thereof; (d) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (e) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and, (f) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. In this preferred aspect of the method, the organism is genetically modified to affect the activity of the PKS system (described in detail above). Preferred host cells for genetic modification related to the PUFA PKS system of the invention are described above.

In the method of production of desired bioactive compounds of the present invention, a genetically modified microorganism is cultured or grown in a suitable medium, under conditions effective to produce the bioactive compound. An appropriate, or effective, medium refers to any medium in which a genetically modified microorganism of the present invention, when cultured, is capable of producing the desired product. Such a medium is typically an aqueous medium comprising assimilable carbon, nitrogen and phosphate sources. Such a medium can also include appropriate

salts, minerals, metals and other nutrients. Microorganisms of the present invention can be cultured in conventional fermentation bioreactors. The microorganisms can be cultured by any fermentation process which includes, but is not limited to, batch, fed-batch, cell recycle, and continuous fermentation. Preferred growth conditions for potential host microorganisms according to the present invention are well known in the art. The desired bioactive molecules produced by the genetically modified microorganism can be recovered from the fermentation medium using conventional separation and purification techniques. For example, the fermentation medium can be filtered or centrifuged to remove microorganisms, cell debris and other particulate matter, and the product can be recovered from the cell-free supernatant by conventional methods, such as, for example, ion exchange, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization and crystallization. Alternatively, microorganisms producing the desired compound, or extracts and various fractions thereof, can be used without removal of the microorganism components from the product.

In the method for production of desired bioactive compounds of the present invention, a genetically modified plant is cultured in a fermentation medium or grown in a suitable medium such as soil. An appropriate, or effective, fermentation medium has been discussed in detail above. A suitable growth medium for higher plants includes any growth medium for plants, including, but not limited to, soil, sand, any other particulate media that support root growth (e.g. vermiculite, perlite, etc.) or Hydroponic culture, as well as suitable light, water and nutritional supplements which optimize the growth of the higher plant. The genetically modified plants of the present invention are engineered to produce significant quantities of the desired product through the activity of the PKS system that is genetically modified according to the present invention. The compounds can be recovered through purification processes which extract the compounds from the plant. In a preferred embodiment, the compound is recovered by harvesting the plant. In this embodiment, the plant can be consumed in its natural state or further processed into consumable products.

As described above, a genetically modified microorganism useful in the present invention can, in one aspect, endogenously contain and express a PUFA PKS system, and the genetic modification can be a genetic modification of one or more of the functional domains of the endogenous PUFA PKS system, whereby the modification has some effect on the activity of the PUFA PKS system. In another aspect, such an organism can endogenously contain and express a PUFA PKS system, and the genetic modification can be an introduction of at least one exogenous nucleic acid sequence (e.g., a recombinant nucleic acid molecule), wherein the exogenous nucleic acid sequence encodes at least one biologically active domain or protein from a second PKS system and/or a protein that affects the activity of said PUFA PKS system (e.g., a phosphopantetheinyl transferases (PPTase), discussed below). In yet another aspect, the organism does not necessarily endogenously (naturally) contain a PUFA PKS system, but is genetically modified to introduce at least one recombinant nucleic acid molecule encoding an amino acid sequence having the biological activity of at least one domain of a PUFA PKS system. In this aspect, PUFA PKS activity is affected by introducing or increasing PUFA PKS activity in the organism. Various embodiments associated with each of these aspects have been discussed in detail above.

In one embodiment of the method to produce bioactive compounds, the genetic modification changes at least one product produced by the endogenous PKS system, as compared to a wild-type organism.

In another embodiment, the organism endogenously expresses a PKS system comprising the at least one biologically active domain of the PUFA PKS system, and the genetic modification comprises transfection of the organism with a recombinant nucleic acid molecule selected from the group consisting of: a recombinant nucleic acid molecule encoding at least one biologically active domain from a second PKS system and a recombinant nucleic acid molecule encoding a protein that affects the activity of the PUFA PKS system. In this embodiment, the genetic modification preferably changes at least one product produced by the endogenous PKS system, as compared to a wild-type organism. A second PKS system can include another PUFA PKS system (bacterial or non-bacterial), a type I PKS system, a type II PKS system, and/or a modular PKS system. Examples of proteins that affect the activity of a PKS system have been described above (e.g., PPTase).

In another embodiment, the organism is genetically modified by transfection with a recombinant nucleic acid molecule encoding the at least one domain of the polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system. Such recombinant nucleic acid molecules have been described in detail previously herein.

In another embodiment, the organism endogenously expresses a non-bacterial PUFA PKS system, and the genetic modification comprises substitution of a domain from a different PKS system for a nucleic acid sequence encoding at least one domain of the non-bacterial PUFA PKS system. In another embodiment, the organism endogenously expresses a non-bacterial PUFA PKS system that has been modified by transfecting the organism with a recombinant nucleic acid molecule encoding a protein that regulates the chain length of fatty acids produced by the PUFA PKS system. In one aspect, the recombinant nucleic acid molecule encoding a protein that regulates the chain length of fatty acids replaces a nucleic acid sequence encoding a chain length factor in the non-bacterial PUFA PKS system. In another aspect, the protein that regulates the chain length of fatty acids produced by the PUFA PKS system is a chain length factor. In another aspect, the protein that regulates the chain length of fatty acids produced by the PUFA PKS system is a chain length factor that directs the synthesis of C20 units.

In another embodiment, the organism expresses a non-bacterial PUFA PKS system comprising a genetic modification in a domain selected from the group consisting of a domain encoding β-hydroxy acyl-ACP dehydrase (DH) and a domain encoding β-ketoacyl-ACP synthase (KS), wherein the modification alters the ratio of long chain fatty acids produced by the PUFA PKS system as compared to in the absence of the modification. In one aspect of this embodiment, the modification is selected from the group consisting of a deletion of all or a part of the domain, a substitution of a homologous domain from a different organism for the domain, and a mutation of the domain.

In another embodiment, the organism expresses a non-bacterial PUFA PKS system comprising a modification in an enoyl-ACP reductase (ER) domain, wherein the modification results in the production of a different compound as compared to in the absence of the modification. In one aspect of this embodiment, the modification is selected from the group consisting of a deletion of all or a part of the ER domain, a substitution of an ER domain from a different organism for the ER domain, and a mutation of the ER domain.

In one embodiment of the method to produce a bioactive molecule, the organism produces a polyunsaturated fatty acid

(PUFA) profile that differs from the naturally occurring organism without a genetic modification.

Many other genetic modifications useful for producing bioactive molecules will be apparent to those of skill in the art, given the present disclosure, and various other modifications have been discussed previously herein. The present invention contemplates any genetic modification related to a PUFA PKS system as described herein which results in the production of a desired bioactive molecule.

Bioactive molecules, according to the present invention, include any molecules (compounds, products, etc.) that have a biological activity, and that can be produced by a PKS system that comprises at least one amino acid sequence having a biological activity of at least one functional domain of a non-bacterial PUFA PKS system as described herein. Such bioactive molecules can include, but are not limited to: a polyunsaturated fatty acid (PUFA), an anti-inflammatory formulation, a chemotherapeutic agent, an active excipient, an osteoporosis drug, an anti-depressant, an anti-convulsant, an anti-*Heliobactor pylori* drug, a drug for treatment of neurodegenerative disease, a drug for treatment of degenerative liver disease, an antibiotic, and a cholesterol lowering formulation. One advantage of the non-bacterial PUFA PKS system of the present invention is the ability of such a system to introduce carbon-carbon double bonds in the cis configuration, and molecules including a double bond at every third carbon. This ability can be utilized to produce a variety of compounds.

Preferably, bioactive compounds of interest are produced by the genetically modified microorganism in an amount that is greater than about 0.05%, and preferably greater than about 0.1%, and more preferably greater than about 0.25%, and more preferably greater than about 0.5%, and more preferably greater than about 0.75%, and more preferably greater than about 1%, and more preferably greater than about 2.5%, and more preferably greater than about 5%, and more preferably greater than about 10%, and more preferably greater than about 15%, and even more preferably greater than about 20% of the dry weight of the microorganism. For lipid compounds, preferably, such compounds are produced in an amount that is greater than about 5% of the dry weight of the microorganism. For other bioactive compounds, such as antibiotics or compounds that are synthesized in smaller amounts, those strains possessing such compounds at of the dry weight of the microorganism are identified as predictably containing a novel PKS system of the type described above. In some embodiments, particular bioactive molecules (compounds) are secreted by the microorganism, rather than accumulating. Therefore, such bioactive molecules are generally recovered from the culture medium and the concentration of molecule produced will vary depending on the microorganism and the size of the culture.

One embodiment of the present invention relates to a method to modify an endproduct containing at least one fatty acid, comprising adding to said endproduct an oil produced by a recombinant host cell that expresses at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The PUFA PKS system is any non-bacterial PUFA PKS system, and preferably, is selected from the group of: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS system from a microorganism identified by the novel screening method disclosed herein; (c) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and biologically active fragments thereof; (d) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (e) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and, (f) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system. Variations of these nucleic acid sequences have been described in detail above.

Preferably, the endproduct is selected from the group consisting of a food, a dietary supplement, a pharmaceutical formulation, a humanized animal milk, and an infant formula. Suitable pharmaceutical formulations include, but are not limited to, an anti-inflammatory formulation, a chemotherapeutic agent, an active excipient, an osteoporosis drug, an anti-depressant, an anti-convulsant, an anti-*Heliobactor pylori* drug, a drug for treatment of neurodegenerative disease, a drug for treatment of degenerative liver disease, an antibiotic, and a cholesterol lowering formulation. In one embodiment, the endproduct is used to treat a condition selected from the group consisting of: chronic inflammation, acute inflammation, gastrointestinal disorder, cancer, cachexia, cardiac restenosis, neurodegenerative disorder, degenerative disorder of the liver, blood lipid disorder, osteoporosis, osteoarthritis, autoimmune disease, preeclampsia, preterm birth, age related maculopathy, pulmonary disorder, and peroxisomal disorder.

Suitable food products include, but are not limited to, fine bakery wares, bread and rolls, breakfast cereals, processed and unprocessed cheese, condiments (ketchup, mayonnaise, etc.), dairy products (milk, yogurt), puddings and gelatine desserts, carbonated drinks, teas, powdered beverage mixes, processed fish products, fruit-based drinks, chewing gum, hard confectionery, frozen dairy products, processed meat products, nut and nut-based spreads, pasta, processed poultry products, gravies and sauces, potato chips and other chips or crisps, chocolate and other confectionery, soups and soup mixes, soya based products (milks, drinks, creams, whiteners), vegetable oil-based spreads, and vegetable-based drinks.

Yet another embodiment of the present invention relates to a method to produce a humanized animal milk. This method includes the steps of genetically modifying milk-producing cells of a milk-producing animal with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding at least one biologically active domain of a PUFA PKS system. The PUFA PKS system is a non-bacterial PUFA PKS system, and preferably, the at least one domain of the PUFA PKS system is encoded by a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding at least one domain of a polyunsaturated fatty acid (PUFA) polyketide synthase (PKS) system from a Thraustochytrid microorganism; (b) a nucleic acid sequence encoding at least one domain of a PUFA PKS sys-

tem from a microorganism identified by the novel screening method described previously herein; (c) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, and biologically active fragments thereof; (d) a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and biologically active fragments thereof; (e) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to at least 500 consecutive amino acids of an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:6; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system; and/or (f) a nucleic acid sequence encoding an amino acid sequence that is at least about 60% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, and SEQ ID NO:32; wherein the amino acid sequence has a biological activity of at least one domain of a PUFA PKS system.

Methods to genetically modify a host cell and to produce a genetically modified non-human, milk-producing animal, are known in the art. Examples of host animals to modify include cattle, sheep, pigs, goats, yaks, etc., which are amenable to genetic manipulation and cloning for rapid expansion of a transgene expressing population. For animals, PKS-like transgenes can be adapted for expression in target organelles, tissues and body fluids through modification of the gene regulatory regions. Of particular interest is the production of PUFAs in the breast milk of the host animal.

The following examples are provided for the purpose of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

The following example describes the further analysis of PKS related sequences from *Schizochytrium.*

The present inventors have sequenced the genomic DNA including the entire length of all three open reading frames (Orfs) in the *Schizochytrium* PUFA PKS system using the general methods outlined in Examples 8 and 9 from PCT Publication No. WO 0042195 and U.S. application Ser. No. 09/231,899. The biologically active domains in the *Schizochytrium* PKS proteins are depicted graphically in FIG. **1**. The domain structure of the *Schizochytrium* PUFA PKS system is described more particularly as follows.

Open Reading Frame A (OrfA):

The complete nucleotide sequence for OrfA is represented herein as SEQ ID NO:1. OrfA is a 8730 nucleotide sequence (not including the stop codon) which encodes a 2910 amino acid sequence, represented herein as SEQ ID NO:2. Within OrfA are twelve domains:

(a) one β-keto acyl-ACP synthase (KS) domain;

(b) one malonyl-CoA:ACP acyltransferase (MAT) domain;

(c) nine acyl carrier protein (ACP) domains;

(d) one ketoreductase (KR) domain.

The domains contained within OrfA have been determined based on:

(1) results of an analysis with Pfam program (Pfam is a database of multiple alignments of protein domains or conserved protein regions. The alignments represent some evolutionary conserved structure that has implications for the protein's function. Profile hidden Markov models (profile HMMs) built from the Pfam alignments can be very useful for automatically recognizing that a new protein belongs to an existing protein family, even if the homology is weak. Unlike standard pairwise alignment methods (e.g. BLAST, FASTA), Pfam HMMs deal sensibly with multidomain proteins. The reference provided for the Pfam version used is: Bateman A, Birney E, Cerruti L, Durbin R, Etwiller L, Eddy S R, Griffiths-Jones S, Howe K L, Marshall M, Sonnhammer E L (2002) Nucleic Acids Research 30(1):276-280); and/or

(2) homology comparison to bacterial PUFA-PKS systems (e.g., *Shewanella*) using a BLAST 2.0 Basic BLAST homology search using blastp for amino acid searches with standard default parameters, wherein the query sequence is filtered for low complexity regions by default (described in Altschul, S. F., Madden, T. L., Schääffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402, incorporated herein by reference in its entirety).

Sequences provided for individual domains are believed to contain the full length of the sequence encoding a functional domain, and may contain additional flanking sequence within the Orf.

ORFA-KS

The first domain in OrfA is a KS domain, also referred to herein as ORFA-KS. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 40 of SEQ ID NO:1 (OrfA) to an ending point of between about positions 1428 and 1500 of SEQ ID NO:1. The nucleotide sequence containing the sequence encoding the ORFA-KS domain is represented herein as SEQ ID NO:7 (positions 1-1500 of SEQ ID NO:1). The amino acid sequence containing the KS domain spans from a starting point of between about positions 1 and 14 of SEQ ID NO:2 (ORFA) to an ending point of between about positions 476 and 500 of SEQ ID NO:2. The amino acid sequence containing the ORFA-KS domain is represented herein as SEQ ID NO:8 (positions 1-500 of SEQ ID NO:2). It is noted that the ORFA-KS domain contains an active site motif: DXAC* (*acyl binding site C_{215}).

ORFA-MAT

The second domain in OrfA is a MAT domain, also referred to herein as ORFA-MAT. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1723 and 1798 of SEQ ID NO:1 (OrfA) to an ending point of between about positions 2805 and 3000 of SEQ ID NO:1. The nucleotide sequence containing the sequence encoding the ORFA-MAT domain is represented herein as SEQ ID NO:9 (positions 1723-3000 of SEQ ID NO:1). The amino acid sequence containing the MAT domain spans from a starting point of between about positions 575 and 600 of SEQ ID NO:2 (ORFA) to an ending point of between about positions 935 and 1000 of SEQ ID NO:2. The amino acid sequence containing the ORFA-MAT domain is represented herein as SEQ ID NO:10 (positions 575-1000 of SEQ ID NO:2). It is noted that the ORFA-MAT domain contains an active site motif: GHS*XG (*acyl binding site S_{706}), represented herein as SEQ ID NO:11.

ORFA-ACP#1-9

Domains 3-11 of OrfA are nine tandem ACP domains, also referred to herein as ORFA-ACP (the first domain in the sequence is ORFA-ACP1, the second domain is ORFA-ACP2, the third domain is ORFA-ACP3, etc.). The first ACP domain, ORFA-ACP1, is contained within the nucleotide sequence spanning from about position 3343 to about position 3600 of SEQ ID NO:1 (OrfA). The nucleotide sequence containing the sequence encoding the ORFA-ACP1 domain is represented herein as SEQ ID NO:12 (positions 3343-3600 of SEQ ID NO:1). The amino acid sequence containing the first ACP domain spans from about position 1115 to about position 1200 of SEQ ID NO:2. The amino acid sequence containing the ORFA-ACP1 domain is represented herein as SEQ ID NO:13 (positions 1115-1200 of SEQ ID NO:2). It is noted that the ORFA-ACP1 domain contains an active site motif: LGIDS* (*pantetheine binding motif S_{1157}), represented herein by SEQ ID NO:14. The nucleotide and amino acid sequences of all nine ACP domains are highly conserved and therefore, the sequence for each domain is not represented herein by an individual sequence identifier. However, based on this information, one of skill in the art can readily determine the sequence for each of the other eight ACP domains. The repeat interval for the nine domains is approximately about 110 to about 330 nucleotides of SEQ ID NO:1.

All nine ACP domains together span a region of OrfA of from about position 3283 to about position 6288 of SEQ ID NO:1, which corresponds to amino acid positions of from about 1095 to about 2096 of SEQ ID NO:2. This region includes the linker segments between individual ACP domains. Each of the nine ACP domains contains a pantetheine binding motif LGIDS* (represented herein by SEQ ID NO:14), wherein * is the pantetheine binding site S. At each end of the ACP domain region and between each ACP domain is a region that is highly enriched for proline (P) and alanine (A), which is believed to be a linker region. For example, between ACP domains 1 and 2 is the sequence: APAPVKAAAPAAPVASAPAPA, represented herein as SEQ ID NO:15.

ORFA-KR

Domain 12 in OrfA is a KR domain, also referred to herein as ORFA-KR. This domain is contained within the nucleotide sequence spanning from a starting point of about position 6598 of SEQ ID NO:1 to an ending point of about position 8730 of SEQ ID NO:1. The nucleotide sequence containing the sequence encoding the ORFA-KR domain is represented herein as SEQ ID NO:17 (positions 6598-8730 of SEQ ID NO:1). The amino acid sequence containing the KR domain spans from a starting point of about position 2200 of SEQ ID NO:2 (ORFA) to an ending point of about position 2910 of SEQ ID NO:2. The amino acid sequence containing the ORFA-KR domain is represented herein as SEQ ID NO:18 (positions 2200-2910 of SEQ ID NO:2). Within the KR domain is a core region with homology to short chain aldehyde-dehydrogenases (KR is a member of this family). This core region spans from about position 7198 to about position 7500 of SEQ ID NO:1, which corresponds to amino acid positions 2400-2500 of SEQ ID NO:2.

Open Reading Frame B (OrfB):

The complete nucleotide sequence for OrfB is represented herein as SEQ ID NO:3. OrfB is a 6177 nucleotide sequence (not including the stop codon) which encodes a 2059 amino acid sequence, represented herein as SEQ ID NO:4. Within OrfB are four domains:

(a) β-keto acyl-ACP synthase (KS) domain;

(b) one chain length factor (CLF) domain;

(c) one acyl transferase (AT) domain;

(d) one enoyl ACP-reductase (ER) domain.

The domains contained within ORFB have been determined based on: (1) results of an analysis with Pfam program, described above; and/or (2) homology comparison to bacterial PUFA-PKS systems (e.g., *Shewanella*) using a BLAST 2.0 Basic BLAST homology search, also described above. Sequences provided for individual domains are believed to contain the full length of the sequence encoding a functional domain, and may contain additional flanking sequence within the Orf.

ORFB-KS

The first domain in OrfB is a KS domain, also referred to herein as ORFB-KS. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 43 of SEQ ID NO:3 (Orf)B) to an ending point of between about positions 1332 and 1350 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-KS domain is represented herein as SEQ ID NO:19 (positions 1-1350 of SEQ ID NO:3). The amino acid sequence containing the KS domain spans from a starting point of between about positions 1 and 15 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 444 and 450 of SEQ ID NO:4. The amino acid sequence containing the ORFB-KS domain is represented herein as SEQ ID NO:20 (positions 1-450 of SEQ ID NO:4). It is noted that the ORFB-KS domain contains an active site motif: DXAC* (*acyl binding site C_{196}).

ORFB-CLF

The second domain in OrfB is a CLF domain, also referred to herein as ORFB-CLF. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1378 and 1402 of SEQ ID NO:3 (OrfB) to an ending point of between about positions 2682 and 2700 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-CLF domain is represented herein as SEQ ID NO:21 (positions 1378-2700 of SEQ ID NO:3). The amino acid sequence containing the CLF domain spans from a starting point of between about positions 460 and 468 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 894 and 900 of SEQ ID NO:4. The amino acid sequence containing the ORFB-CLF domain is represented herein as SEQ ID NO:22 (positions 460-900 of SEQ ID NO:4). It is noted that the ORFB-CLF domain contains a KS active site motif without the acyl-binding cysteine.

ORFB-AT

The third domain in OrfB is an AT domain, also referred to herein as ORFB-AT. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 2701 and 3598 of SEQ ID NO:3 (OrfB) to an ending point of between about positions 3975 and 4200 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-AT domain is represented herein as SEQ ID NO:23 (positions 2701-4200 of SEQ ID NO:3). The amino acid sequence containing the AT domain spans from a starting point of between about positions 901 and 1200 of SEQ ID NO:4 (ORFB) to an ending point of between about positions 1325 and 1400 of SEQ ID NO:4. The amino acid sequence containing the ORFB-AT domain is represented herein as SEQ ID NO:24 (positions 901-1400 of SEQ ID NO:4). It is noted that the ORFB-AT domain contains an AT active site motif of G×S*×G (*acyl binding site S_{1140}).

ORFB-ER

The fourth domain in OrfB is an ER domain, also referred to herein as ORFB-ER. This domain is contained within the nucleotide sequence spanning from a starting point of about position 4648 of SEQ ID NO:3 (OrfB) to an ending point of about position 6177 of SEQ ID NO:3. The nucleotide sequence containing the sequence encoding the ORFB-ER domain is represented herein as SEQ ID NO:25 (positions 4648-6177 of SEQ ID NO:3). The amino acid sequence containing the ER domain spans from a starting point of about position 1550 of SEQ ID NO:4 (ORFB) to an ending point of about position 2059 of SEQ ID NO:4. The amino acid sequence containing the ORFB-ER domain is represented herein as SEQ ID NO:26 (positions 1550-2059 of SEQ ID NO:4).

Open Reading Frame C (OrfC):

The complete nucleotide sequence for OrfC is represented herein as SEQ ID NO:5. OrfC is a 4509 nucleotide sequence (not including the stop codon) which encodes a 1503 amino acid sequence, represented herein as SEQ ID NO:6. Within OrfC are three domains:

(a) two FabA-like β-hydroxy acyl-ACP dehydrase (DH) domains;

(b) one enoyl ACP-reductase (ER) domain.

The domains contained within ORFC have been determined based on: (1) results of an analysis with Pfam program, described above; and/or (2) homology comparison to bacterial PUFA-PKS systems (e.g., *Shewanella*) using a BLAST 2.0 Basic BLAST homology search, also described above. Sequences provided for individual domains are believed to contain the full length of the sequence encoding a functional domain, and may contain additional flanking sequence within the Orf.

ORFC-DH1

The first domain in OrfC is a DH domain, also referred to herein as ORFC-DH1. This is one of two DH domains in OrfC, and therefore is designated DH1. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1 and 778 of SEQ ID NO:5 (OrfC) to an ending point of between about positions 1233 and 1350 of SEQ ID NO:5. The nucleotide sequence containing the sequence encoding the ORFC-DH1 domain is represented herein as SEQ ID NO:27 (positions 1-1350 of SEQ ID NO:5). The amino acid sequence containing the DH1 domain spans from a starting point of between about positions 1 and 260 of SEQ ID NO:6 (ORFC) to an ending point of between about positions 411 and 450 of SEQ ID NO:6. The amino acid sequence containing the ORFC-DH1 domain is represented herein as SEQ ID NO:28 (positions 1-450 of SEQ ID NO:6).

ORFC-DH2

The second domain in OrfC is a DH domain, also referred to herein as ORFC-DH2. This is the second of two DH domains in OrfC, and therefore is designated DH2. This domain is contained within the nucleotide sequence spanning from a starting point of between about positions 1351 and 2437 of SEQ ID NO:5 (OrfC) to an ending point of between about positions 2607 and 2850 of SEQ ID NO:5. The nucleotide sequence containing the sequence encoding the ORFC-DH2 domain is represented herein as SEQ ID NO:29 (positions 1351-2850 of SEQ ID NO:5). The amino acid sequence containing the DH2 domain spans from a starting point of between about positions 451 and 813 of SEQ ID NO:6 (ORFC) to an ending point of between about positions 869 and 950 of SEQ ID NO:6. The amino acid sequence containing the ORFC-DH2 domain is represented herein as SEQ ID NO:30 (positions 451-950 of SEQ ID NO:6).

ORFC-ER

The third domain in OrfC is an ER domain, also referred to herein as ORFC-ER. This domain is contained within the nucleotide sequence spanning from a starting point of about position 2998 of SEQ ID NO:5 (OrfC) to an ending point of about position 4509 of SEQ ID NO:5. The nucleotide sequence containing the sequence encoding the ORFC-ER domain is represented herein as SEQ ID NO:31 (positions 2998-4509 of SEQ ID NO:5). The amino acid sequence containing the ER domain spans from a starting point of about position 1000 of SEQ ID NO:6 (ORFC) to an ending point of about position 1502 of SEQ ID NO:6. The amino acid sequence containing the ORFC-ER domain is represented herein as SEQ ID NO:32 (positions 1000-1502 of SEQ ID NO:6).

Example 2

The following example describes the use of the screening process of the present invention to identify three other non-bacterial organisms comprising a PUFA PKS system according to the present invention.

Thraustochytrium sp. 23B (ATCC 20892) was cultured according to the screening method described in U.S. Provisional Application Ser. No. 60/298,796 and as described in detail herein.

The biorational screen (using shake flask cultures) developed for detecting microorganisms containing PUFA producing PKS systems is as follows:

Two mL of a culture of the strain/microorganism to be tested is placed in 250 mL baffled shake flask with 50 mL culture media (aerobic treatment) and another 2 mL of culture of the same strain is placed in a 250 mL non-baffled shake flask with 200 mL culture medium (anoxic treatment). Both flasks are placed on a shaker table at 200 rpm. After 48-72 hr of culture time, the cultures are harvested by centrifugation and the cells analyzed for fatty acid methyl esters via gas chromatography to determine the following data for each culture: (1) fatty acid profile; (2) PUFA content; (3) fat content (estimated as amount total fatty acids (TFA)).

These data are then analyzed asking the following five questions:

Selection Criteria: Low O_2/Anoxic Flask vs. Aerobic Flask (Yes/No)

(1) Did the DHA (or other PUFA content) (as % FAME) stay about the same or preferably increase in the low oxygen culture compared to the aerobic culture?

(2) Is C14:0+C16:0+C16:1 greater than about 40% TFA in the anoxic culture?

(3) Is there very little (>1% as FAME) or no precursors (C18:3n-3+C18:2n-6+C18:3n-6) to the conventional oxygen dependent elongase/desaturase pathway in the anoxic culture?

(4) Did fat content (as amount total fatty acids/cell dry weight) increase in the low oxygen culture compared to the aerobic culture?

(5) Did DHA (or other PUFA content) increase as % cell dry weight in the low oxygen culture compared to the aerobic culture?

If first three questions are answered yes, there is a good indication that the strain contains a PKS genetic system for making long chain PUFAs. The more questions that are answered yes (preferably the first three questions must be answered yes), the stronger the indication that the strain con-

tains such a PKS genetic system. If all five questions are answered yes, then there is a very strong indication that the strain contains a PKS genetic system for making long chain PUFAs.

Following the method outlined above, a frozen vial of *Thraustochytrium* sp. 23B (ATCC 20892) was used to inoculate a 250 mL shake flask containing 50 mL of RCA medium. The culture was shaken on a shaker table (200 rpm) for 72 hr at 25° C. RCA medium contains the following:

RCA Medium	
Deionized water	1000 mL
Reef Crystals ® sea salts	40 g/L
Glucose	20 g/L
Monosodium glutamate (MSG)	20 g/L
Yeast extract	1 g/L
PII metals*	5 mL/L
Vitamin mix*	1 mL/L
pH	7.0

*PII metal mix and vitamin mix are same as those outlined in U.S. Pat. No. 5,130,742, incorporated herein by reference in its entirety.

25 mL of the 72 hr old culture was then used to inoculate another 250 mL shake flask containing 50 mL of low nitrogen RCA medium (10 g/L MSG instead of 20 g/L) and the other 25 mL of culture was used to inoculate a 250 mL shake flask containing 175 mL of low-nitrogen RCA medium. The two flasks were then placed on a shaker table (200 rpm) for 72 hr at 25° C. The cells were then harvested via centrifugation and dried by lyophilization. The dried cells were analyzed for fat content and fatty acid profile and content using standard gas chromatograph procedures (such as those outlined in U.S. Pat. No. 5,130,742).

The screening results for *Thraustochytrium* 23B were as follows:

Did DHA as % FAME increase?	Yes (38 -> 44%)
C14:0 + C16:0 + C16:1 greater than about 40% TFA?	Yes (44%)
No C18:3(n − 3) or C18:3(n − 6)?	Yes (0%)
Did fat content increase?	Yes (2-fold increase)
Did DHA (or other HUFA content increase)?	Yes (2.3-fold increase)

The results, especially the significant increase in DHA content (as % FAME) under low oxygen conditions, conditions, strongly indicates the presence of a PUFA producing PKS system in this strain of *Thraustochytrium.*

In order to provide additional data confirming the presence of a PUFA PKS system, southern blot of *Thraustochytrium* 23B was conducted using PKS probes from *Schizochytrium* strain 20888, a strain which has already been determined to contain a PUFA producing PKS system (i.e., SEQ ID Nos:1-32 described above). Fragments of *Thraustochytrium* 23B genomic DNA which are homologous to hybridization probes from PKS PUFA synthesis genes were detected using the Southern blot technique. *Thraustochytrium* 23B genomic DNA was digested with either ClaI or KpnI restriction endonucleases, separated by agarose gel electrophoresis (0.7% agarose, in standard Tris-Acetate-EDTA buffer), and blotted to a Schleicher & Schuell Nytran Supercharge membrane by capillary transfer. Two digoxigenin labeled hybridization probes were used—one specific for the Enoyl Reductase (ER) region of *Schizochytrium* PKS Orf B (nucleotides 5012-5511 of Orf B; SEQ ID NO:3), and the other specific for a conserved region at the beginning of *Schizochytrium* PKS Orf C (nucleotides 76-549 of OrfC; SEQ ID NO:5).

The OrfB-ER probe detected an approximately 13 kb ClaI fragment and an approximately 3.6 kb KpnI fragment in the *Thraustochytrium* 23B genomic DNA. The OrfC probe detected an approximately 7.5 kb ClaI fragment and an approximately 4.6 kb KpnI fragment in the *Thraustochytrium* 23B genomic DNA.

Finally, a recombinant genomic library, consisting of DNA fragments from *Thraustochytrium* 23B genomic DNA inserted into vector lambda FIX II (Stratagene), was screened using digoxigenin labeled probes corresponding to the following segments of *Schizochytrium* 20888 PUFA-PKS genes: nucleotides 7385-7879 of Orf A (SEQ ID NO:1), nucleotides 5012-5511 of Orf B (SEQ ID NO:3), and nucleotides 76-549 of Orf C (SEQ ID NO:5). Each of these probes detected positive plaques from the *Thraustochytrium* 23B library, indicating extensive homology between the *Schizochytrium* PUFA-PKS genes and the genes of *Thraustochytrium* 23B.

In summary, these results demonstrate that *Thraustochytrium* 23B genomic DNA contains sequences that are homologous to PKS genes from *Schizochytrium* 20888.

This Thraustochytrid microorganism is encompassed herein as an additional sources of these genes for use in the embodiments above.

Thraustochytrium 23B (ATCC 20892) is significantly different from *Schizochytrium* sp. (ATCC 20888) in its fatty acid profile. *Thraustochytrium* 23B can have DHA:DPA(n-6) ratios as high as 14:1 compared to only 2-3:1 in *Schizochytrium* (ATCC 20888). *Thraustochytrium* 23B can also have higher levels of C20:5(n-3). Analysis of the domains in the PUFA PKS system of *Thraustochytrium* 23B in comparison to the known *Schizochytrium* PUFA PKS system should provide us with key information on how to modify these domains to influence the ratio and types of PUFA produced using these systems.

The screening method described above has been utilized the identify other potential candidate strains containing a PUFA PKS system. Two additional strains that have been identified by the present inventors to have PUFA PKS systems are *Schizochytrium limacium* (SR21) Honda & Yokochi (IFO32693) and *Ulkenia* (BP-5601). Both were screened as above but in N2 media (glucose: 60 g/L; KH_2PO_4: 4.0 μl; yeast extract: 1.0 g/L; corn steep liquor: 1 mL/L; NH_4NO_3: 1.0 g/L; artificial sea salts (Reef Crystals): 20 g/L; all above concentrations mixed in deionized water). For both the *Schizochytrium* and *Ulkenia* strains, the answers to the first three screen questions discussed above for *Thraustochytrium* 23B was yes (*Schizochytrium*—DHA % FAME 32->41% aerobic vs anoxic, 58% 14:0/16:0/16:1, 0% precursors) and (*Ulkenia*—DHA % FAME 28->44% aerobic vs anoxic, 63% 14:0/16:0/16:1, 0% precursors), indicating that these strains are good candidates for containing a PUFA PKS system. Negative answers were obtained for the final two questions for each strain: fat decreased from 61% dry wt to 22% dry weight, and DHA from 21-9% dry weight in *S. limacium* and fat decreased from 59 to 21% dry weight in *Ulkenia* and DHA from 16% to 9% dry weight. These Thraustochytrid microorganisms are also claimed herein as additional sources of the genes for use in the embodiments above.

Example 3

The following example demonstrates that DHA and DPA synthesis in *Schizochytrium* does not involve membrane-bound desaturases or fatty acid elongation enzymes like those

described for other eukaryotes (Parker-Barnes et al., 2000, supra; Shanklin et al., 1998, supra).

Schizochytrium accumulates large quantities of triacylglycerols rich in DHA and docosapentaenoic acid (DPA; 22:5ω6); e.g., 30% DHA+DPA by dry weight. In eukaryotes that synthesize 20- and 22-carbon PUFAs by an elongation/desaturation pathway, the pools of 18-, 20- and 22-carbon intermediates are relatively large so that in vivo labeling experiments using [^{14}C]-acetate reveal clear precursor-product kinetics for the predicted intermediates. Furthermore, radiolabeled intermediates provided exogenously to such organisms are converted to the final PUFA products.

[1-^{14}C]acetate was supplied to a 2-day-old culture as a single pulse at zero time. Samples of cells were then harvested by centrifugation and the lipids were extracted. In addition, [1-^{14}C]acetate uptake by the cells was estimated by measuring the radioactivity of the sample before and after centrifugation. Fatty acid methyl esters derived from the total cell lipids were separated by $AgNO_3$-TLC (solvent, hexane:diethyl ether:acetic acid, 70:30:2 by volume). The identity of the fatty acid bands was verified by gas chromatography, and the radioactivity in them was measured by scintillation counting. Results showed that [1-^{14}C]-acetate was rapidly taken up by *Schizochytrium* cells and incorporated into fatty acids, but at the shortest labeling time (1 min) DHA contained 31% of the label recovered in fatty acids and this percentage remained essentially unchanged during the 10-15 min of [^{14}C]-acetate incorporation and the subsequent 24 hours of culture growth (data not shown). Similarly, DPA represented 10% of the label throughout the experiment. There is no evidence for a precursor-product relationship between 16- or 18-carbon fatty acids and the 22-carbon polyunsaturated fatty acids. These results are consistent with rapid synthesis of DHA from [^{14}C]-acetate involving very small (possibly enzyme-bound) pools of intermediates.

Next, cells were disrupted in 100 mM phosphate buffer (pH 7.2), containing 2 mM DTT, 2 mM EDTA, and 10% glycerol, by vortexing with glass beads. The cell-free homogenate was centrifuged at 100,000 g for 1 hour. Equivalent aliquots of total homogenate, pellet (H-S pellet), and supernatant (H-S super) fractions were incubated in homogenization buffer supplemented with 20 μM acetyl-CoA, 100 μM [1-^{14}C]malonyl-CoA (0.9 Gbq/mol), 2 mM NADH, and 2 mM NADPH for 60 min at 25° C. Assays were extracted and fatty acid methyl esters were prepared and separated as described above before detection of radioactivity with an Instantimager (Packard Instruments, Meriden, Conn.). Results showed that a cell-free homogenate derived from *Schizochytrium* cultures incorporated [1-^{14}C]-malonyl-CoA into DHA, DPA, and saturated fatty acids (data not shown). The same biosynthetic activities were retained by a 100,000×g supernatant fraction but were not present in the membrane pellet. These data contrast with those obtained during assays of the bacterial enzymes (see Metz et al., 2001, supra) and may indicate use of a different (soluble) acyl acceptor molecule. Thus, DHA and DPA synthesis in *Schizochytrium* does not involve membrane-bound desaturases or fatty acid elongation enzymes like those described for other eukaryotes.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

SEQUENCE LISTING

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<160> NUMBER OF SEQ ID NOS: 37

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<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(8730)

<400> SEQUENCE: 1

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1               5                   10                  15

att gcc atc atc ggc atg tcg gcc atc ctc ccc tgc ggc acg acc gtg       96
Ile Ala Ile Ile Gly Met Ser Ala Ile Leu Pro Cys Gly Thr Thr Val
            20                  25                  30

cgc gag tcg tgg gag acc atc cgc gcc ggc atc gac tgc ctg tcg gat      144
Arg Glu Ser Trp Glu Thr Ile Arg Ala Gly Ile Asp Cys Leu Ser Asp
        35                  40                  45

ctc ccc gag gac cgc gtc gac gtg acg gcg tac ttt gac ccc gtc aag      192
Leu Pro Glu Asp Arg Val Asp Val Thr Ala Tyr Phe Asp Pro Val Lys
    50                  55                  60

acc acc aag gac aag atc tac tgc aag cgc ggt ggc ttc att ccc gag      240
Thr Thr Lys Asp Lys Ile Tyr Cys Lys Arg Gly Gly Phe Ile Pro Glu
65                  70                  75                  80

tac gac ttt gac gcc cgc gag ttc gga ctc aac atg ttc cag atg gag      288
Tyr Asp Phe Asp Ala Arg Glu Phe Gly Leu Asn Met Phe Gln Met Glu
                85                  90                  95
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Asp Ser Asp Ala Asn Gln Thr Ile Ser Leu Leu Lys Val Lys Glu Ala
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ctc cag gac gcc ggc atc gac gcc ctc ggc aag gaa aag aag aac atc      384
Leu Gln Asp Ala Gly Ile Asp Ala Leu Gly Lys Glu Lys Lys Asn Ile
        115                 120                 125

ggc tgc gtg ctc ggc att ggc ggc ggc caa aag tcc agc cac gag ttc      432
Gly Cys Val Leu Gly Ile Gly Gly Gly Gln Lys Ser Ser His Glu Phe
    130                 135                 140

tac tcg cgc ctt aat tat gtt gtc gtg gag aag gtc ctc cgc aag atg      480
Tyr Ser Arg Leu Asn Tyr Val Val Val Glu Lys Val Leu Arg Lys Met
145                 150                 155                 160

ggc atg ccc gag gag gac gtc aag gtc gcc gtc gaa aag tac aag gcc      528
Gly Met Pro Glu Glu Asp Val Lys Val Ala Val Glu Lys Tyr Lys Ala
                165                 170                 175

aac ttc ccc gag tgg cgc ctc gac tcc ttc cct ggc ttc ctc ggc aac      576
Asn Phe Pro Glu Trp Arg Leu Asp Ser Phe Pro Gly Phe Leu Gly Asn
            180                 185                 190

gtc acc gcc ggt cgc tgc acc aac acc ttc aac ctc gac ggc atg aac      624
Val Thr Ala Gly Arg Cys Thr Asn Thr Phe Asn Leu Asp Gly Met Asn
        195                 200                 205

tgc gtt gtc gac gcc gca tgc gcc tcg tcc ctc atc gcc gtc aag gtc      672
Cys Val Val Asp Ala Ala Cys Ala Ser Ser Leu Ile Ala Val Lys Val
    210                 215                 220

gcc atc gac gag ctg ctc tac ggt gac tgc gac atg atg gtc acc ggt      720
Ala Ile Asp Glu Leu Leu Tyr Gly Asp Cys Asp Met Met Val Thr Gly
225                 230                 235                 240

gcc acc tgc acg gat aac tcc atc ggc atg tac atg gcc ttc tcc aag      768
Ala Thr Cys Thr Asp Asn Ser Ile Gly Met Tyr Met Ala Phe Ser Lys
                245                 250                 255

acc ccc gtg ttc tcc acg gac ccc agc gtg cgc gcc tac gac gaa aag      816
Thr Pro Val Phe Ser Thr Asp Pro Ser Val Arg Ala Tyr Asp Glu Lys
            260                 265                 270

aca aag ggc atg ctc atc ggc gag ggc tcc gcc atg ctc gtc ctc aag      864
Thr Lys Gly Met Leu Ile Gly Glu Gly Ser Ala Met Leu Val Leu Lys
        275                 280                 285

cgc tac gcc gac gcc gtc cgc gac ggc gat gag atc cac gct gtt att      912
Arg Tyr Ala Asp Ala Val Arg Asp Gly Asp Glu Ile His Ala Val Ile
    290                 295                 300

cgc ggc tgc gcc tcc tcc agt gat ggc aag gcc gcc ggc atc tac acg      960
Arg Gly Cys Ala Ser Ser Ser Asp Gly Lys Ala Ala Gly Ile Tyr Thr
305                 310                 315                 320

ccc acc att tcg ggc cag gag gag gcc ctc cgc cgc gcc tac aac cgc     1008
Pro Thr Ile Ser Gly Gln Glu Glu Ala Leu Arg Arg Ala Tyr Asn Arg
                325                 330                 335

gcc tgt gtc gac ccg gcc acc gtc act ctc gtc gag ggt cac ggc acc     1056
Ala Cys Val Asp Pro Ala Thr Val Thr Leu Val Glu Gly His Gly Thr
            340                 345                 350

ggt act ccc gtt ggc gac cgc atc gag ctc acc gcc ttg cgc aac ctc     1104
Gly Thr Pro Val Gly Asp Arg Ile Glu Leu Thr Ala Leu Arg Asn Leu
        355                 360                 365

ttt gac aag gcc tac ggc gag ggc aac acc gaa aag gtc gct gtg ggc     1152
Phe Asp Lys Ala Tyr Gly Glu Gly Asn Thr Glu Lys Val Ala Val Gly
    370                 375                 380

agc atc aag tcc agc atc ggc cat ctc aag gcc gtc gcc ggt ctc gcc     1200
Ser Ile Lys Ser Ser Ile Gly His Leu Lys Ala Val Ala Gly Leu Ala
385                 390                 395                 400

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Gly Met Ile Lys Val Ile Met Ala Leu Lys His Lys Thr Leu Pro Gly
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            420                 425                 430

aac gag tcc tcg ctc tac att aac acc atg aac cgc ccc tgg ttc ccg      1344
Asn Glu Ser Ser Leu Tyr Ile Asn Thr Met Asn Arg Pro Trp Phe Pro
        435                 440                 445

ccc cct ggt gtg ccc cgc cgc gcc ggc att tcg agc ttt ggc ttt ggt      1392
Pro Pro Gly Val Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly
    450                 455                 460

ggc gcc aac tac cac gcc gtc ctc gag gag gcc gag ccc gag cac acg      1440
Gly Ala Asn Tyr His Ala Val Leu Glu Glu Ala Glu Pro Glu His Thr
465                 470                 475                 480

acc gcg tac cgc ctc aac aag cgc ccg cag ccc gtg ctc atg atg gcc      1488
Thr Ala Tyr Arg Leu Asn Lys Arg Pro Gln Pro Val Leu Met Met Ala
            485                 490                 495

gcc acg ccc gcg gcc ctc cag tcg ctc tgc gag gcc cag ctc aag gag      1536
Ala Thr Pro Ala Ala Leu Gln Ser Leu Cys Glu Ala Gln Leu Lys Glu
            500                 505                 510

ttc gag gcc gcc atc aag gag aac gag acc gtc aag aac acc gcc tac      1584
Phe Glu Ala Ala Ile Lys Glu Asn Glu Thr Val Lys Asn Thr Ala Tyr
        515                 520                 525

atc aag tgc gtc aag ttc ggc gag cag ttc aaa ttc cct ggc tcc atc      1632
Ile Lys Cys Val Lys Phe Gly Glu Gln Phe Lys Phe Pro Gly Ser Ile
    530                 535                 540

ccg gcc aca aac gcg cgc ctc ggc ttc ctc gtc aag gat gct gag gat      1680
Pro Ala Thr Asn Ala Arg Leu Gly Phe Leu Val Lys Asp Ala Glu Asp
545                 550                 555                 560

gcc tgc tcc acc ctc cgt gcc atc tgc gcc caa ttc gcc aag gat gtc      1728
Ala Cys Ser Thr Leu Arg Ala Ile Cys Ala Gln Phe Ala Lys Asp Val
                565                 570                 575

acc aag gag gcc tgg cgc ctc ccc cgc gag ggc gtc agc ttc cgc gcc      1776
Thr Lys Glu Ala Trp Arg Leu Pro Arg Glu Gly Val Ser Phe Arg Ala
            580                 585                 590

aag ggc atc gcc acc aac ggc gct gtc gcc gcg ctc ttc tcc ggc cag      1824
Lys Gly Ile Ala Thr Asn Gly Ala Val Ala Ala Leu Phe Ser Gly Gln
        595                 600                 605

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Gly Ala Gln Tyr Thr His Met Phe Ser Glu Val Ala Met Asn Trp Pro
    610                 615                 620

cag ttc cgc cag agc att gcc gcc atg gac gcc gcc cag tcc aag gtc      1920
Gln Phe Arg Gln Ser Ile Ala Ala Met Asp Ala Ala Gln Ser Lys Val
625                 630                 635                 640

gct gga agc gac aag gac ttt gag cgc gtc tcc cag gtc ctc tac ccg      1968
Ala Gly Ser Asp Lys Asp Phe Glu Arg Val Ser Gln Val Leu Tyr Pro
            645                 650                 655

cgc aag ccg tac gag cgt gag ccc gag cag aac ccc aag aag atc tcc      2016
Arg Lys Pro Tyr Glu Arg Glu Pro Glu Gln Asn Pro Lys Lys Ile Ser
            660                 665                 670

ctc acc gcc tac tcg cag ccc tcg acc ctg gcc tgc gct ctc ggt gcc      2064
Leu Thr Ala Tyr Ser Gln Pro Ser Thr Leu Ala Cys Ala Leu Gly Ala
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Phe Glu Ile Phe Lys Glu Ala Gly Phe Thr Pro Asp Phe Ala Ala Gly
    690                 695                 700

cat tcg ctc ggt gag ttc gcc gcc ctc tac gcc gcg ggc tgc gtc gac      2160
His Ser Leu Gly Glu Phe Ala Ala Leu Tyr Ala Ala Gly Cys Val Asp
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Gly Lys Asp Ala Pro Ala Thr Pro Lys Gly Cys Met Ala Ala Val Ile
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ggc ccc aac gcc gag aac atc aag gtc cag gcc gcc aac gtc tgg ctc      2304
Gly Pro Asn Ala Glu Asn Ile Lys Val Gln Ala Ala Asn Val Trp Leu
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ggc aac tcc aac tcg cct tcg cag acc gtc atc acc ggc tcc gtc gaa      2352
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    770                 775                 780

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785                 790                 795                 800

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Val Pro Leu Ala Cys Glu Ser Ala Phe His Ser Pro Gln Met Glu Asn
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Ala Ser Ser Ala Phe Lys Asp Val Ile Ser Lys Val Ser Phe Arg Thr
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ccc aag gcc gag acc aag ctc ttc agc aac gtc tct ggc gag acc tac      2544
Pro Lys Ala Glu Thr Lys Leu Phe Ser Asn Val Ser Gly Glu Thr Tyr
        835                 840                 845

ccc acg gac gcc cgc gag atg ctt acg cag cac atg acc agc agc gtc      2592
Pro Thr Asp Ala Arg Glu Met Leu Thr Gln His Met Thr Ser Ser Val
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aag ttc ctc acc cag gtc cgc aac atg cac cag gcc ggt gcg cgc atc      2640
Lys Phe Leu Thr Gln Val Arg Asn Met His Gln Ala Gly Ala Arg Ile
865                 870                 875                 880

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Phe Val Glu Phe Gly Pro Lys Gln Val Leu Ser Lys Leu Val Ser Glu
                885                 890                 895

acc ctc aag gat gac ccc tcg gtt gtc acc gtc tct gtc aac ccg gcc      2736
Thr Leu Lys Asp Asp Pro Ser Val Val Thr Val Ser Val Asn Pro Ala
            900                 905                 910

tcg ggc acg gat tcg gac atc cag ctc cgc gac gcg gcc gtc cag ctc      2784
Ser Gly Thr Asp Ser Asp Ile Gln Leu Arg Asp Ala Ala Val Gln Leu
        915                 920                 925

gtt gtc gct ggc gtc aac ctt cag ggc ttt gac aag tgg gac gcc ccc      2832
Val Val Ala Gly Val Asn Leu Gln Gly Phe Asp Lys Trp Asp Ala Pro
    930                 935                 940

gat gcc acc cgc atg cag gcc atc aag aag aag cgc act acc ctc cgc      2880
Asp Ala Thr Arg Met Gln Ala Ile Lys Lys Lys Arg Thr Thr Leu Arg
945                 950                 955                 960

ctt tcg gcc gcc acc tac gtc tcg gac aag acc aag aag gtc cgc gac      2928
Leu Ser Ala Ala Thr Tyr Val Ser Asp Lys Thr Lys Lys Val Arg Asp
                965                 970                 975

gcc gcc atg aac gat ggc cgc tgc gtc acc tac ctc aag ggc gcc gca      2976
Ala Ala Met Asn Asp Gly Arg Cys Val Thr Tyr Leu Lys Gly Ala Ala
            980                 985                 990

ccg ctc atc aag gcc ccg gag ccc  gtt gtc gac gag gcc  gcc aag cgc    3024
Pro Leu Ile Lys Ala Pro Glu Pro  Val Val Asp Glu Ala  Ala Lys Arg
        995                 1000                 1005

gag gcc  gag cgt ctc cag aag  gag ctt cag gat gcc  cag cgc cag      3069
Glu Ala  Glu Arg Leu Gln Lys  Glu Leu Gln Asp Ala  Gln Arg Gln
    1010                 1015                 1020

ctc gac  gac gcc aag cgc gcc  gcc gcc gag gcc aac  tcc aag ctc      3114
Leu Asp  Asp Ala Lys Arg Ala  Ala Ala Glu Ala Asn  Ser Lys Leu
    1025                 1030                 1035
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gcc gct  gcc aag gag gag gcc  aag acc gcc gct gct  tcg gcc aag      3159
Ala Ala  Ala Lys Glu Glu Ala  Lys Thr Ala Ala Ala  Ser Ala Lys
    1040                1045                1050

ccc gca  gtt gac act gct gtt  gtc gaa aag cat cgt  gcc atc ctc      3204
Pro Ala  Val Asp Thr Ala Val  Val Glu Lys His Arg  Ala Ile Leu
    1055                1060                1065

aag tcc  atg ctc gcg gag ctc  gat ggc tac gga tcg  gtc gac gct      3249
Lys Ser  Met Leu Ala Glu Leu  Asp Gly Tyr Gly Ser  Val Asp Ala
    1070                1075                1080

tct tcc  ctc cag cag cag cag  cag cag cag acg gcc  ccc gcc ccg      3294
Ser Ser  Leu Gln Gln Gln Gln  Gln Gln Gln Thr Ala  Pro Ala Pro
    1085                1090                1095

gtc aag  gct gct gcg cct gcc  gcc ccc gtt gcc tcg  gcc cct gcc      3339
Val Lys  Ala Ala Ala Pro Ala  Ala Pro Val Ala Ser  Ala Pro Ala
    1100                1105                1110

ccg gct  gtc tcg aac gag ctt  ctt gag aag gcc gag  act gtc gtc      3384
Pro Ala  Val Ser Asn Glu Leu  Leu Glu Lys Ala Glu  Thr Val Val
    1115                1120                1125

atg gag  gtc ctc gcc gcc aag  acc ggc tac gag acc  gac atg atc      3429
Met Glu  Val Leu Ala Ala Lys  Thr Gly Tyr Glu Thr  Asp Met Ile
    1130                1135                1140

gag gct  gac atg gag ctc gag  acc gag ctc ggc att  gac tcc atc      3474
Glu Ala  Asp Met Glu Leu Glu  Thr Glu Leu Gly Ile  Asp Ser Ile
    1145                1150                1155

aag cgt  gtc gag atc ctc tcc  gag gtc cag gcc atg  ctc aat gtc      3519
Lys Arg  Val Glu Ile Leu Ser  Glu Val Gln Ala Met  Leu Asn Val
    1160                1165                1170

gag gcc  aag gat gtc gat gcc  ctc agc cgc act cgc  act gtt ggt      3564
Glu Ala  Lys Asp Val Asp Ala  Leu Ser Arg Thr Arg  Thr Val Gly
    1175                1180                1185

gag gtt  gtc aac gcc atg aag  gcc gag atc gct ggc  agc tct gcc      3609
Glu Val  Val Asn Ala Met Lys  Ala Glu Ile Ala Gly  Ser Ser Ala
    1190                1195                1200

ccg gcg  cct gct gcc gct gct  ccg gct ccg gcc aag  gct gcc cct      3654
Pro Ala  Pro Ala Ala Ala Ala  Pro Ala Pro Ala Lys  Ala Ala Pro
    1205                1210                1215

gcc gcc  gct gcg cct gct gtc  tcg aac gag ctt ctc  gag aag gcc      3699
Ala Ala  Ala Ala Pro Ala Val  Ser Asn Glu Leu Leu  Glu Lys Ala
    1220                1225                1230

gag acc  gtc gtc atg gag gtc  ctc gcc gcc aag act  ggc tac gag      3744
Glu Thr  Val Val Met Glu Val  Leu Ala Ala Lys Thr  Gly Tyr Glu
    1235                1240                1245

act gac  atg atc gag tcc gac  atg gag ctc gag act  gag ctc ggc      3789
Thr Asp  Met Ile Glu Ser Asp  Met Glu Leu Glu Thr  Glu Leu Gly
    1250                1255                1260

att gac  tcc atc aag cgt gtc  gag atc ctc tcc gag  gtt cag gcc      3834
Ile Asp  Ser Ile Lys Arg Val  Glu Ile Leu Ser Glu  Val Gln Ala
    1265                1270                1275

atg ctc  aac gtc gag gcc aag  gac gtc gac gct ctc  agc cgc act      3879
Met Leu  Asn Val Glu Ala Lys  Asp Val Asp Ala Leu  Ser Arg Thr
    1280                1285                1290

cgc act  gtg ggt gag gtc gtc  aac gcc atg aag gct  gag atc gct      3924
Arg Thr  Val Gly Glu Val Val  Asn Ala Met Lys Ala  Glu Ile Ala
    1295                1300                1305

ggt ggc  tct gcc ccg gcg cct  gcc gcc gct gcc cca  ggt ccg gct      3969
Gly Gly  Ser Ala Pro Ala Pro  Ala Ala Ala Ala Pro  Gly Pro Ala
    1310                1315                1320

gct gcc  gcc cct gcg cct gcc  gcc gcc gcc cct gct  gtc tcg aac      4014
Ala Ala  Ala Pro Ala Pro Ala  Ala Ala Ala Pro Ala  Val Ser Asn
    1325                1330                1335
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gag ctt  ctt gag aag gcc gag  acc gtc gtc atg gag  gtc ctc gcc      4059
Glu Leu  Leu Glu Lys Ala Glu  Thr Val Val Met Glu  Val Leu Ala
    1340                 1345                 1350

gcc aag  act ggc tac gag act  gac atg atc gag tcc  gac atg gag      4104
Ala Lys  Thr Gly Tyr Glu Thr  Asp Met Ile Glu Ser  Asp Met Glu
    1355                 1360                 1365

ctc gag  acc gag ctc ggc att  gac tcc atc aag cgt  gtc gag att      4149
Leu Glu  Thr Glu Leu Gly Ile  Asp Ser Ile Lys Arg  Val Glu Ile
    1370                 1375                 1380

ctc tcc  gag gtc cag gcc atg  ctc aac gtc gag gcc  aag gac gtc      4194
Leu Ser  Glu Val Gln Ala Met  Leu Asn Val Glu Ala  Lys Asp Val
    1385                 1390                 1395

gac gct  ctc agc cgc acc cgc  act gtt ggc gag gtc  gtc gat gcc      4239
Asp Ala  Leu Ser Arg Thr Arg  Thr Val Gly Glu Val  Val Asp Ala
    1400                 1405                 1410

atg aag  gcc gag atc gct ggt  ggc tct gcc ccg gcg  cct gcc gcc      4284
Met Lys  Ala Glu Ile Ala Gly  Gly Ser Ala Pro Ala  Pro Ala Ala
    1415                 1420                 1425

gct gct  cct gct ccg gct gct  gcc gcc cct gcg cct  gcc gcc cct      4329
Ala Ala  Pro Ala Pro Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Pro
    1430                 1435                 1440

gcg cct  gct gtc tcg agc gag  ctt ctc gag aag gcc  gag act gtc      4374
Ala Pro  Ala Val Ser Ser Glu  Leu Leu Glu Lys Ala  Glu Thr Val
    1445                 1450                 1455

gtc atg  gag gtc ctc gcc gcc  aag act ggc tac gag  act gac atg      4419
Val Met  Glu Val Leu Ala Ala  Lys Thr Gly Tyr Glu  Thr Asp Met
    1460                 1465                 1470

atc gag  tcc gac atg gag ctc  gag acc gag ctc ggc  att gac tcc      4464
Ile Glu  Ser Asp Met Glu Leu  Glu Thr Glu Leu Gly  Ile Asp Ser
    1475                 1480                 1485

atc aag  cgt gtc gag att ctc  tcc gag gtc cag gcc  atg ctc aac      4509
Ile Lys  Arg Val Glu Ile Leu  Ser Glu Val Gln Ala  Met Leu Asn
    1490                 1495                 1500

gtc gag  gcc aag gac gtc gac  gct ctc agc cgc acc  cgc act gtt      4554
Val Glu  Ala Lys Asp Val Asp  Ala Leu Ser Arg Thr  Arg Thr Val
    1505                 1510                 1515

ggc gag  gtc gtc gat gcc atg  aag gcc gag atc gct  ggt ggc tct      4599
Gly Glu  Val Val Asp Ala Met  Lys Ala Glu Ile Ala  Gly Gly Ser
    1520                 1525                 1530

gcc ccg  gcg cct gcc gcc gct  gct cct gct ccg gct  gct gcc gcc      4644
Ala Pro  Ala Pro Ala Ala Ala  Ala Pro Ala Pro Ala  Ala Ala Ala
    1535                 1540                 1545

cct gcg  cct gcc gcc cct gcg  cct gcc gcc cct gcg  cct gct gtc      4689
Pro Ala  Pro Ala Ala Pro Ala  Pro Ala Ala Pro Ala  Pro Ala Val
    1550                 1555                 1560

tcg agc  gag ctt ctc gag aag  gcc gag act gtc gtc  atg gag gtc      4734
Ser Ser  Glu Leu Leu Glu Lys  Ala Glu Thr Val Val  Met Glu Val
    1565                 1570                 1575

ctc gcc  gcc aag act ggc tac  gag act gac atg att  gag tcc gac      4779
Leu Ala  Ala Lys Thr Gly Tyr  Glu Thr Asp Met Ile  Glu Ser Asp
    1580                 1585                 1590

atg gag  ctc gag acc gag ctc  ggc att gac tcc atc  aag cgt gtc      4824
Met Glu  Leu Glu Thr Glu Leu  Gly Ile Asp Ser Ile  Lys Arg Val
    1595                 1600                 1605

gag att  ctc tcc gag gtt cag  gcc atg ctc aac gtc  gag gcc aag      4869
Glu Ile  Leu Ser Glu Val Gln  Ala Met Leu Asn Val  Glu Ala Lys
    1610                 1615                 1620

gac gtc  gac gct ctc agc cgc  act cgc act gtt ggt  gag gtc gtc      4914
Asp Val  Asp Ala Leu Ser Arg  Thr Arg Thr Val Gly  Glu Val Val
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    1625                1630                1635

gat gcc  atg aag gct gag atc  gct ggc agc tcc gcc  tcg gcg cct      4959
Asp Ala  Met Lys Ala Glu Ile  Ala Gly Ser Ser Ala  Ser Ala Pro
    1640                1645                1650

gcc gcc  gct gct cct gct ccg  gct gct gcc gct cct  gcg ccc gct      5004
Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Ala Ala Pro  Ala Pro Ala
    1655                1660                1665

gcc gcc  gcc cct gct gtc tcg  aac gag ctt ctc gag  aaa gcc gag      5049
Ala Ala  Ala Pro Ala Val Ser  Asn Glu Leu Leu Glu  Lys Ala Glu
    1670                1675                1680

act gtc  gtc atg gag gtc ctc  gcc gcc aag act ggc  tac gag act      5094
Thr Val  Val Met Glu Val Leu  Ala Ala Lys Thr Gly  Tyr Glu Thr
    1685                1690                1695

gac atg  atc gag tcc gac atg  gag ctc gag act gag  ctc ggc att      5139
Asp Met  Ile Glu Ser Asp Met  Glu Leu Glu Thr Glu  Leu Gly Ile
    1700                1705                1710

gac tcc  atc aag cgt gtc gag  atc ctc tcc gag gtt  cag gcc atg      5184
Asp Ser  Ile Lys Arg Val Glu  Ile Leu Ser Glu Val  Gln Ala Met
    1715                1720                1725

ctc aac  gtc gag gcc aag gac  gtc gat gcc ctc agc  cgc acc cgc      5229
Leu Asn  Val Glu Ala Lys Asp  Val Asp Ala Leu Ser  Arg Thr Arg
    1730                1735                1740

act gtt  ggc gag gtt gtc gat  gcc atg aag gcc gag  atc gct ggt      5274
Thr Val  Gly Glu Val Val Asp  Ala Met Lys Ala Glu  Ile Ala Gly
    1745                1750                1755

ggc tct  gcc ccg gcg cct gcc  gcc gct gcc cct gct  ccg gct gcc      5319
Gly Ser  Ala Pro Ala Pro Ala  Ala Ala Ala Pro Ala  Pro Ala Ala
    1760                1765                1770

gcc gcc  cct gct gtc tcg aac  gag ctt ctc gag aag  gcc gag act      5364
Ala Ala  Pro Ala Val Ser Asn  Glu Leu Leu Glu Lys  Ala Glu Thr
    1775                1780                1785

gtc gtc  atg gag gtc ctc gcc  gcc aag act ggc tac  gag acc gac      5409
Val Val  Met Glu Val Leu Ala  Ala Lys Thr Gly Tyr  Glu Thr Asp
    1790                1795                1800

atg atc  gag tcc gac atg gag  ctc gag acc gag ctc  ggc att gac      5454
Met Ile  Glu Ser Asp Met Glu  Leu Glu Thr Glu Leu  Gly Ile Asp
    1805                1810                1815

tcc atc  aag cgt gtc gag att  ctc tcc gag gtt cag  gcc atg ctc      5499
Ser Ile  Lys Arg Val Glu Ile  Leu Ser Glu Val Gln  Ala Met Leu
    1820                1825                1830

aac gtc  gag gcc aag gac gtc  gat gct ctc agc cgc  act cgc act      5544
Asn Val  Glu Ala Lys Asp Val  Asp Ala Leu Ser Arg  Thr Arg Thr
    1835                1840                1845

gtt ggc  gag gtc gtc gat gcc  atg aag gct gag atc  gcc ggc agc      5589
Val Gly  Glu Val Val Asp Ala  Met Lys Ala Glu Ile  Ala Gly Ser
    1850                1855                1860

tcc gcc  ccg gcg cct gcc gcc  gct gct cct gct ccg  gct gct gcc      5634
Ser Ala  Pro Ala Pro Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Ala
    1865                1870                1875

gct cct  gcg ccc gct gcc gct  gcc cct gct gtc tcg  agc gag ctt      5679
Ala Pro  Ala Pro Ala Ala Ala  Ala Pro Ala Val Ser  Ser Glu Leu
    1880                1885                1890

ctc gag  aag gcc gag acc gtc  gtc atg gag gtc ctc  gcc gcc aag      5724
Leu Glu  Lys Ala Glu Thr Val  Val Met Glu Val Leu  Ala Ala Lys
    1895                1900                1905

act ggc  tac gag act gac atg  att gag tcc gac atg  gag ctc gag      5769
Thr Gly  Tyr Glu Thr Asp Met  Ile Glu Ser Asp Met  Glu Leu Glu
    1910                1915                1920

act gag  ctc ggc att gac tcc  atc aag cgt gtc gag  atc ctc tcc      5814
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Thr Glu  Leu Gly Ile Asp Ser  Ile Lys Arg Val Glu  Ile Leu Ser
    1925                1930                1935

gag gtt  cag gcc atg ctc aac  gtc gag gcc aag gac  gtc gat gcc      5859
Glu Val  Gln Ala Met Leu Asn  Val Glu Ala Lys Asp  Val Asp Ala
    1940                1945                1950

ctc agc  cgc acc cgc act gtt  ggc gag gtt gtc gat  gcc atg aag      5904
Leu Ser  Arg Thr Arg Thr Val  Gly Glu Val Val Asp  Ala Met Lys
    1955                1960                1965

gcc gag  atc gct ggt ggc tct  gcc ccg gcg cct gcc  gcc gct gcc      5949
Ala Glu  Ile Ala Gly Gly Ser  Ala Pro Ala Pro Ala  Ala Ala Ala
    1970                1975                1980

cct gct  ccg gct gcc gcc gcc  cct gct gtc tcg aac  gag ctt ctt      5994
Pro Ala  Pro Ala Ala Ala Ala  Pro Ala Val Ser Asn  Glu Leu Leu
    1985                1990                1995

gag aag  gcc gag acc gtc gtc  atg gag gtc ctc gcc  gcc aag act      6039
Glu Lys  Ala Glu Thr Val Val  Met Glu Val Leu Ala  Ala Lys Thr
    2000                2005                2010

ggc tac  gag acc gac atg atc  gag tcc gac atg gag  ctc gag acc      6084
Gly Tyr  Glu Thr Asp Met Ile  Glu Ser Asp Met Glu  Leu Glu Thr
    2015                2020                2025

gag ctc  ggc att gac tcc atc  aag cgt gtc gag att  ctc tcc gag      6129
Glu Leu  Gly Ile Asp Ser Ile  Lys Arg Val Glu Ile  Leu Ser Glu
    2030                2035                2040

gtt cag  gcc atg ctc aac gtc  gag gcc aag gac gtc  gac gct ctc      6174
Val Gln  Ala Met Leu Asn Val  Glu Ala Lys Asp Val  Asp Ala Leu
    2045                2050                2055

agc cgc  act cgc act gtt ggc  gag gtc gtc gat gcc  atg aag gct      6219
Ser Arg  Thr Arg Thr Val Gly  Glu Val Val Asp Ala  Met Lys Ala
    2060                2065                2070

gag atc  gct ggt ggc tct gcc  ccg gcg cct gcc gcc  gct gct cct      6264
Glu Ile  Ala Gly Gly Ser Ala  Pro Ala Pro Ala Ala  Ala Ala Pro
    2075                2080                2085

gcc tcg  gct ggc gcc gcg cct  gcg gtc aag att gac  tcg gtc cac      6309
Ala Ser  Ala Gly Ala Ala Pro  Ala Val Lys Ile Asp  Ser Val His
    2090                2095                2100

ggc gct  gac tgt gat gat ctt  tcc ctg atg cac gcc  aag gtg gtt      6354
Gly Ala  Asp Cys Asp Asp Leu  Ser Leu Met His Ala  Lys Val Val
    2105                2110                2115

gac atc  cgc cgc ccg gac gag  ctc atc ctg gag cgc  ccc gag aac      6399
Asp Ile  Arg Arg Pro Asp Glu  Leu Ile Leu Glu Arg  Pro Glu Asn
    2120                2125                2130

cgc ccc  gtt ctc gtt gtc gat  gac ggc agc gag ctc  acc ctc gcc      6444
Arg Pro  Val Leu Val Val Asp  Asp Gly Ser Glu Leu  Thr Leu Ala
    2135                2140                2145

ctg gtc  cgc gtc ctc ggc gcc  tgc gcc gtt gtc ctg  acc ttt gag      6489
Leu Val  Arg Val Leu Gly Ala  Cys Ala Val Val Leu  Thr Phe Glu
    2150                2155                2160

ggt ctc  cag ctc gct cag cgc  gct ggt gcc gct gcc  atc cgc cac      6534
Gly Leu  Gln Leu Ala Gln Arg  Ala Gly Ala Ala Ala  Ile Arg His
    2165                2170                2175

gtg ctc  gcc aag gat ctt tcc  gcg gag agc gcc gag  aag gcc atc      6579
Val Leu  Ala Lys Asp Leu Ser  Ala Glu Ser Ala Glu  Lys Ala Ile
    2180                2185                2190

aag gag  gcc gag cag cgc ttt  ggc gct ctc ggc ggc  ttc atc tcg      6624
Lys Glu  Ala Glu Gln Arg Phe  Gly Ala Leu Gly Gly  Phe Ile Ser
    2195                2200                2205

cag cag  gcg gag cgc ttc gag  ccc gcc gaa atc ctc  ggc ttc acg      6669
Gln Gln  Ala Glu Arg Phe Glu  Pro Ala Glu Ile Leu  Gly Phe Thr
    2210                2215                2220
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ctc atg  tgc gcc aag ttc gcc  aag gct tcc ctc tgc  acg gct gtg      6714
Leu Met  Cys Ala Lys Phe Ala  Lys Ala Ser Leu Cys  Thr Ala Val
    2225                 2230                 2235

gct ggc  ggc cgc ccg gcc ttt  atc ggt gtg gcg cgc  ctt gac ggc      6759
Ala Gly  Gly Arg Pro Ala Phe  Ile Gly Val Ala Arg  Leu Asp Gly
    2240                 2245                 2250

cgc ctc  gga ttc act tcg cag  ggc act tct gac gcg  ctc aag cgt      6804
Arg Leu  Gly Phe Thr Ser Gln  Gly Thr Ser Asp Ala  Leu Lys Arg
    2255                 2260                 2265

gcc cag  cgt ggt gcc atc ttt  ggc ctc tgc aag acc  atc ggc ctc      6849
Ala Gln  Arg Gly Ala Ile Phe  Gly Leu Cys Lys Thr  Ile Gly Leu
    2270                 2275                 2280

gag tgg  tcc gag tct gac gtc  ttt tcc cgc ggc gtg  gac att gct      6894
Glu Trp  Ser Glu Ser Asp Val  Phe Ser Arg Gly Val  Asp Ile Ala
    2285                 2290                 2295

cag ggc  atg cac ccc gag gat  gcc gcc gtg gcg att  gtg cgc gag      6939
Gln Gly  Met His Pro Glu Asp  Ala Ala Val Ala Ile  Val Arg Glu
    2300                 2305                 2310

atg gcg  tgc gct gac att cgc  att cgc gag gtc ggc  att ggc gca      6984
Met Ala  Cys Ala Asp Ile Arg  Ile Arg Glu Val Gly  Ile Gly Ala
    2315                 2320                 2325

aac cag  cag cgc tgc acg atc  cgt gcc gcc aag ctc  gag acc ggc      7029
Asn Gln  Gln Arg Cys Thr Ile  Arg Ala Ala Lys Leu  Glu Thr Gly
    2330                 2335                 2340

aac ccg  cag cgc cag atc gcc  aag gac gac gtg ctg  ctc gtt tct      7074
Asn Pro  Gln Arg Gln Ile Ala  Lys Asp Asp Val Leu  Leu Val Ser
    2345                 2350                 2355

ggc ggc  gct cgc ggc atc acg  cct ctt tgc atc cgg  gag atc acg      7119
Gly Gly  Ala Arg Gly Ile Thr  Pro Leu Cys Ile Arg  Glu Ile Thr
    2360                 2365                 2370

cgc cag  atc gcg ggc ggc aag  tac att ctg ctt ggc  cgc agc aag      7164
Arg Gln  Ile Ala Gly Gly Lys  Tyr Ile Leu Leu Gly  Arg Ser Lys
    2375                 2380                 2385

gtc tct  gcg agc gaa ccg gca  tgg tgc gct ggc atc  act gac gag      7209
Val Ser  Ala Ser Glu Pro Ala  Trp Cys Ala Gly Ile  Thr Asp Glu
    2390                 2395                 2400

aag gct  gtg caa aag gct gct  acc cag gag ctc aag  cgc gcc ttt      7254
Lys Ala  Val Gln Lys Ala Ala  Thr Gln Glu Leu Lys  Arg Ala Phe
    2405                 2410                 2415

agc gct  ggc gag ggc ccc aag  ccc acg ccc cgc gct  gtc act aag      7299
Ser Ala  Gly Glu Gly Pro Lys  Pro Thr Pro Arg Ala  Val Thr Lys
    2420                 2425                 2430

ctt gtg  ggc tct gtt ctt ggc  gct cgc gag gtg cgc  agc tct att      7344
Leu Val  Gly Ser Val Leu Gly  Ala Arg Glu Val Arg  Ser Ser Ile
    2435                 2440                 2445

gct gcg  att gaa gcg ctc ggc  ggc aag gcc atc tac  tcg tcg tgc      7389
Ala Ala  Ile Glu Ala Leu Gly  Gly Lys Ala Ile Tyr  Ser Ser Cys
    2450                 2455                 2460

gac gtg  aac tct gcc gcc gac  gtg gcc aag gcc gtg  cgc gat gcc      7434
Asp Val  Asn Ser Ala Ala Asp  Val Ala Lys Ala Val  Arg Asp Ala
    2465                 2470                 2475

gag tcc  cag ctc ggt gcc cgc  gtc tcg ggc atc gtt  cat gcc tcg      7479
Glu Ser  Gln Leu Gly Ala Arg  Val Ser Gly Ile Val  His Ala Ser
    2480                 2485                 2490

ggc gtg  ctc cgc gac cgt ctc  atc gag aag aag ctc  ccc gac gag      7524
Gly Val  Leu Arg Asp Arg Leu  Ile Glu Lys Lys Leu  Pro Asp Glu
    2495                 2500                 2505

ttc gac  gcc gtc ttt ggc acc  aag gtc acc ggt ctc  gag aac ctc      7569
Phe Asp  Ala Val Phe Gly Thr  Lys Val Thr Gly Leu  Glu Asn Leu
    2510                 2515                 2520
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ctc gcc  gcc gtc gac cgc gcc  aac ctc aag cac atg  gtc ctc ttc      7614
Leu Ala  Ala Val Asp Arg Ala  Asn Leu Lys His Met  Val Leu Phe
    2525                 2530                 2535

agc tcg  ctc gcc ggc ttc cac  ggc aac gtc ggc cag  tct gac tac      7659
Ser Ser  Leu Ala Gly Phe His  Gly Asn Val Gly Gln  Ser Asp Tyr
    2540                 2545                 2550

gcc atg  gcc aac gag gcc ctt  aac aag atg ggc ctc  gag ctc gcc      7704
Ala Met  Ala Asn Glu Ala Leu  Asn Lys Met Gly Leu  Glu Leu Ala
    2555                 2560                 2565

aag gac  gtc tcg gtc aag tcg  atc tgc ttc ggt ccc  tgg gac ggt      7749
Lys Asp  Val Ser Val Lys Ser  Ile Cys Phe Gly Pro  Trp Asp Gly
    2570                 2575                 2580

ggc atg  gtg acg ccg cag ctc  aag aag cag ttc cag  gag atg ggc      7794
Gly Met  Val Thr Pro Gln Leu  Lys Lys Gln Phe Gln  Glu Met Gly
    2585                 2590                 2595

gtg cag  atc atc ccc cgc gag  ggc ggc gct gat acc  gtg gcg cgc      7839
Val Gln  Ile Ile Pro Arg Glu  Gly Gly Ala Asp Thr  Val Ala Arg
    2600                 2605                 2610

atc gtg  ctc ggc tcc tcg ccg  gct gag atc ctt gtc  ggc aac tgg      7884
Ile Val  Leu Gly Ser Ser Pro  Ala Glu Ile Leu Val  Gly Asn Trp
    2615                 2620                 2625

cgc acc  ccg tcc aag aag gtc  ggc tcg gac acc atc  acc ctg cac      7929
Arg Thr  Pro Ser Lys Lys Val  Gly Ser Asp Thr Ile  Thr Leu His
    2630                 2635                 2640

cgc aag  att tcc gcc aag tcc  aac ccc ttc ctc gag  gac cac gtc      7974
Arg Lys  Ile Ser Ala Lys Ser  Asn Pro Phe Leu Glu  Asp His Val
    2645                 2650                 2655

atc cag  ggc cgc cgc gtg ctg  ccc atg acg ctg gcc  att ggc tcg      8019
Ile Gln  Gly Arg Arg Val Leu  Pro Met Thr Leu Ala  Ile Gly Ser
    2660                 2665                 2670

ctc gcg  gag acc tgc ctc ggc  ctc ttc ccc ggc tac  tcg ctc tgg      8064
Leu Ala  Glu Thr Cys Leu Gly  Leu Phe Pro Gly Tyr  Ser Leu Trp
    2675                 2680                 2685

gcc att  gac gac gcc cag ctc  ttc aag ggt gtc act  gtc gac ggc      8109
Ala Ile  Asp Asp Ala Gln Leu  Phe Lys Gly Val Thr  Val Asp Gly
    2690                 2695                 2700

gac gtc  aac tgc gag gtg acc  ctc acc ccg tcg acg  gcg ccc tcg      8154
Asp Val  Asn Cys Glu Val Thr  Leu Thr Pro Ser Thr  Ala Pro Ser
    2705                 2710                 2715

ggc cgc  gtc aac gtc cag gcc  acg ctc aag acc ttt  tcc agc ggc      8199
Gly Arg  Val Asn Val Gln Ala  Thr Leu Lys Thr Phe  Ser Ser Gly
    2720                 2725                 2730

aag ctg  gtc ccg gcc tac cgc  gcc gtc atc gtg ctc  tcc aac cag      8244
Lys Leu  Val Pro Ala Tyr Arg  Ala Val Ile Val Leu  Ser Asn Gln
    2735                 2740                 2745

ggc gcg  ccc ccg gcc aac gcc  acc atg cag ccg ccc  tcg ctc gat      8289
Gly Ala  Pro Pro Ala Asn Ala  Thr Met Gln Pro Pro  Ser Leu Asp
    2750                 2755                 2760

gcc gat  ccg gcg ctc cag ggc  tcc gtc tac gac ggc  aag acc ctc      8334
Ala Asp  Pro Ala Leu Gln Gly  Ser Val Tyr Asp Gly  Lys Thr Leu
    2765                 2770                 2775

ttc cac  ggc ccg gcc ttc cgc  ggc atc gat gac gtg  ctc tcg tgc      8379
Phe His  Gly Pro Ala Phe Arg  Gly Ile Asp Asp Val  Leu Ser Cys
    2780                 2785                 2790

acc aag  agc cag ctt gtg gcc  aag tgc agc gct gtc  ccc ggc tcc      8424
Thr Lys  Ser Gln Leu Val Ala  Lys Cys Ser Ala Val  Pro Gly Ser
    2795                 2800                 2805

gac gcc  gct cgc ggc gag ttt  gcc acg gac act gac  gcc cat gac      8469
Asp Ala  Ala Arg Gly Glu Phe  Ala Thr Asp Thr Asp  Ala His Asp
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    2810                2815                2820

ccc ttc  gtg aac gac ctg gcc  ttt cag gcc atg ctc  gtc tgg gtg      8514
Pro Phe  Val Asn Asp Leu Ala  Phe Gln Ala Met Leu  Val Trp Val
    2825                2830                2835

cgc cgc  acg ctc ggc cag gct  gcg ctc ccc aac tcg  atc cag cgc      8559
Arg Arg  Thr Leu Gly Gln Ala  Ala Leu Pro Asn Ser  Ile Gln Arg
    2840                2845                2850

atc gtc  cag cac cgc ccg gtc  ccg cag gac aag ccc  ttc tac att      8604
Ile Val  Gln His Arg Pro Val  Pro Gln Asp Lys Pro  Phe Tyr Ile
    2855                2860                2865

acc ctc  cgc tcc aac cag tcg  ggc ggt cac tcc cag  cac aag cac      8649
Thr Leu  Arg Ser Asn Gln Ser  Gly Gly His Ser Gln  His Lys His
    2870                2875                2880

gcc ctt  cag ttc cac aac gag  cag ggc gat ctc ttc  att gat gtc      8694
Ala Leu  Gln Phe His Asn Glu  Gln Gly Asp Leu Phe  Ile Asp Val
    2885                2890                2895

cag gct  tcg gtc atc gcc acg  gac agc ctt gcc ttc                   8730
Gln Ala  Ser Val Ile Ala Thr  Asp Ser Leu Ala Phe
    2900                2905                2910


<210> SEQ ID NO 2
<211> LENGTH: 2910
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 2

Met Ala Ala Arg Leu Gln Glu Gln Lys Gly Gly Glu Met Asp Thr Arg
1               5                   10                  15

Ile Ala Ile Ile Gly Met Ser Ala Ile Leu Pro Cys Gly Thr Thr Val
            20                  25                  30

Arg Glu Ser Trp Glu Thr Ile Arg Ala Gly Ile Asp Cys Leu Ser Asp
        35                  40                  45

Leu Pro Glu Asp Arg Val Asp Val Thr Ala Tyr Phe Asp Pro Val Lys
    50                  55                  60

Thr Thr Lys Asp Lys Ile Tyr Cys Lys Arg Gly Gly Phe Ile Pro Glu
65                  70                  75                  80

Tyr Asp Phe Asp Ala Arg Glu Phe Gly Leu Asn Met Phe Gln Met Glu
                85                  90                  95

Asp Ser Asp Ala Asn Gln Thr Ile Ser Leu Leu Lys Val Lys Glu Ala
            100                 105                 110

Leu Gln Asp Ala Gly Ile Asp Ala Leu Gly Lys Glu Lys Lys Asn Ile
        115                 120                 125

Gly Cys Val Leu Gly Ile Gly Gly Gly Gln Lys Ser Ser His Glu Phe
    130                 135                 140

Tyr Ser Arg Leu Asn Tyr Val Val Val Glu Lys Val Leu Arg Lys Met
145                 150                 155                 160

Gly Met Pro Glu Glu Asp Val Lys Val Ala Val Glu Lys Tyr Lys Ala
                165                 170                 175

Asn Phe Pro Glu Trp Arg Leu Asp Ser Phe Pro Gly Phe Leu Gly Asn
            180                 185                 190

Val Thr Ala Gly Arg Cys Thr Asn Thr Phe Asn Leu Asp Gly Met Asn
        195                 200                 205

Cys Val Val Asp Ala Ala Cys Ala Ser Ser Leu Ile Ala Val Lys Val
    210                 215                 220

Ala Ile Asp Glu Leu Leu Tyr Gly Asp Cys Asp Met Met Val Thr Gly
225                 230                 235                 240
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Ala Thr Cys Thr Asp Asn Ser Ile Gly Met Tyr Met Ala Phe Ser Lys
                245                 250                 255

Thr Pro Val Phe Ser Thr Asp Pro Ser Val Arg Ala Tyr Asp Glu Lys
            260                 265                 270

Thr Lys Gly Met Leu Ile Gly Glu Gly Ser Ala Met Leu Val Leu Lys
        275                 280                 285

Arg Tyr Ala Asp Ala Val Arg Asp Gly Asp Glu Ile His Ala Val Ile
    290                 295                 300

Arg Gly Cys Ala Ser Ser Ser Asp Gly Lys Ala Ala Gly Ile Tyr Thr
305                 310                 315                 320

Pro Thr Ile Ser Gly Gln Glu Glu Ala Leu Arg Arg Ala Tyr Asn Arg
                325                 330                 335

Ala Cys Val Asp Pro Ala Thr Val Thr Leu Val Glu Gly His Gly Thr
            340                 345                 350

Gly Thr Pro Val Gly Asp Arg Ile Glu Leu Thr Ala Leu Arg Asn Leu
        355                 360                 365

Phe Asp Lys Ala Tyr Gly Glu Gly Asn Thr Glu Lys Val Ala Val Gly
    370                 375                 380

Ser Ile Lys Ser Ser Ile Gly His Leu Lys Ala Val Ala Gly Leu Ala
385                 390                 395                 400

Gly Met Ile Lys Val Ile Met Ala Leu Lys His Lys Thr Leu Pro Gly
                405                 410                 415

Thr Ile Asn Val Asp Asn Pro Pro Asn Leu Tyr Asp Asn Thr Pro Ile
            420                 425                 430

Asn Glu Ser Ser Leu Tyr Ile Asn Thr Met Asn Arg Pro Trp Phe Pro
        435                 440                 445

Pro Pro Gly Val Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly
    450                 455                 460

Gly Ala Asn Tyr His Ala Val Leu Glu Glu Ala Glu Pro Glu His Thr
465                 470                 475                 480

Thr Ala Tyr Arg Leu Asn Lys Arg Pro Gln Pro Val Leu Met Met Ala
                485                 490                 495

Ala Thr Pro Ala Ala Leu Gln Ser Leu Cys Glu Ala Gln Leu Lys Glu
            500                 505                 510

Phe Glu Ala Ala Ile Lys Glu Asn Glu Thr Val Lys Asn Thr Ala Tyr
        515                 520                 525

Ile Lys Cys Val Lys Phe Gly Glu Gln Phe Lys Phe Pro Gly Ser Ile
    530                 535                 540

Pro Ala Thr Asn Ala Arg Leu Gly Phe Leu Val Lys Asp Ala Glu Asp
545                 550                 555                 560

Ala Cys Ser Thr Leu Arg Ala Ile Cys Ala Gln Phe Ala Lys Asp Val
                565                 570                 575

Thr Lys Glu Ala Trp Arg Leu Pro Arg Glu Gly Val Ser Phe Arg Ala
            580                 585                 590

Lys Gly Ile Ala Thr Asn Gly Ala Val Ala Ala Leu Phe Ser Gly Gln
        595                 600                 605

Gly Ala Gln Tyr Thr His Met Phe Ser Glu Val Ala Met Asn Trp Pro
    610                 615                 620

Gln Phe Arg Gln Ser Ile Ala Ala Met Asp Ala Ala Gln Ser Lys Val
625                 630                 635                 640

Ala Gly Ser Asp Lys Asp Phe Glu Arg Val Ser Gln Val Leu Tyr Pro
                645                 650                 655

Arg Lys Pro Tyr Glu Arg Glu Pro Glu Gln Asn Pro Lys Lys Ile Ser
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            660                 665                 670

Leu Thr Ala Tyr Ser Gln Pro Ser Thr Leu Ala Cys Ala Leu Gly Ala
        675                 680                 685

Phe Glu Ile Phe Lys Glu Ala Gly Phe Thr Pro Asp Phe Ala Ala Gly
    690                 695                 700

His Ser Leu Gly Glu Phe Ala Ala Leu Tyr Ala Ala Gly Cys Val Asp
705                 710                 715                 720

Arg Asp Glu Leu Phe Glu Leu Val Cys Arg Arg Ala Arg Ile Met Gly
                725                 730                 735

Gly Lys Asp Ala Pro Ala Thr Pro Lys Gly Cys Met Ala Ala Val Ile
            740                 745                 750

Gly Pro Asn Ala Glu Asn Ile Lys Val Gln Ala Ala Asn Val Trp Leu
        755                 760                 765

Gly Asn Ser Asn Ser Pro Ser Gln Thr Val Ile Thr Gly Ser Val Glu
    770                 775                 780

Gly Ile Gln Ala Glu Ser Ala Arg Leu Gln Lys Glu Gly Phe Arg Val
785                 790                 795                 800

Val Pro Leu Ala Cys Glu Ser Ala Phe His Ser Pro Gln Met Glu Asn
                805                 810                 815

Ala Ser Ser Ala Phe Lys Asp Val Ile Ser Lys Val Ser Phe Arg Thr
            820                 825                 830

Pro Lys Ala Glu Thr Lys Leu Phe Ser Asn Val Ser Gly Glu Thr Tyr
        835                 840                 845

Pro Thr Asp Ala Arg Glu Met Leu Thr Gln His Met Thr Ser Ser Val
    850                 855                 860

Lys Phe Leu Thr Gln Val Arg Asn Met His Gln Ala Gly Ala Arg Ile
865                 870                 875                 880

Phe Val Glu Phe Gly Pro Lys Gln Val Leu Ser Lys Leu Val Ser Glu
                885                 890                 895

Thr Leu Lys Asp Asp Pro Ser Val Val Thr Val Ser Val Asn Pro Ala
            900                 905                 910

Ser Gly Thr Asp Ser Asp Ile Gln Leu Arg Asp Ala Ala Val Gln Leu
        915                 920                 925

Val Val Ala Gly Val Asn Leu Gln Gly Phe Asp Lys Trp Asp Ala Pro
    930                 935                 940

Asp Ala Thr Arg Met Gln Ala Ile Lys Lys Lys Arg Thr Thr Leu Arg
945                 950                 955                 960

Leu Ser Ala Ala Thr Tyr Val Ser Asp Lys Thr Lys Lys Val Arg Asp
                965                 970                 975

Ala Ala Met Asn Asp Gly Arg Cys Val Thr Tyr Leu Lys Gly Ala Ala
            980                 985                 990

Pro Leu Ile Lys Ala Pro Glu Pro  Val Val Asp Glu Ala  Ala Lys Arg
        995                 1000                1005

Glu Ala  Glu Arg Leu Gln Lys  Glu Leu Gln Asp Ala  Gln Arg Gln
    1010                1015                1020

Leu Asp  Asp Ala Lys Arg Ala  Ala Ala Glu Ala Asn  Ser Lys Leu
    1025                1030                1035

Ala Ala  Ala Lys Glu Glu Ala  Lys Thr Ala Ala Ala  Ser Ala Lys
    1040                1045                1050

Pro Ala  Val Asp Thr Ala Val  Val Glu Lys His Arg  Ala Ile Leu
    1055                1060                1065

Lys Ser  Met Leu Ala Glu Leu  Asp Gly Tyr Gly Ser  Val Asp Ala
    1070                1075                1080
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Ser Ser  Leu Gln Gln Gln Gln  Gln Gln Gln Thr Ala  Pro Ala Pro
    1085                 1090                 1095

Val Lys  Ala Ala Ala Pro Ala  Ala Pro Val Ala Ser  Ala Pro Ala
    1100                 1105                 1110

Pro Ala  Val Ser Asn Glu Leu  Leu Glu Lys Ala Glu  Thr Val Val
    1115                 1120                 1125

Met Glu  Val Leu Ala Ala Lys  Thr Gly Tyr Glu Thr  Asp Met Ile
    1130                 1135                 1140

Glu Ala  Asp Met Glu Leu Glu  Thr Glu Leu Gly Ile  Asp Ser Ile
    1145                 1150                 1155

Lys Arg  Val Glu Ile Leu Ser  Glu Val Gln Ala Met  Leu Asn Val
    1160                 1165                 1170

Glu Ala  Lys Asp Val Asp Ala  Leu Ser Arg Thr Arg  Thr Val Gly
    1175                 1180                 1185

Glu Val  Val Asn Ala Met Lys  Ala Glu Ile Ala Gly  Ser Ser Ala
    1190                 1195                 1200

Pro Ala  Pro Ala Ala Ala Ala  Pro Ala Pro Ala Lys  Ala Ala Pro
    1205                 1210                 1215

Ala Ala  Ala Ala Pro Ala Val  Ser Asn Glu Leu Leu  Glu Lys Ala
    1220                 1225                 1230

Glu Thr  Val Val Met Glu Val  Leu Ala Ala Lys Thr  Gly Tyr Glu
    1235                 1240                 1245

Thr Asp  Met Ile Glu Ser Asp  Met Glu Leu Glu Thr  Glu Leu Gly
    1250                 1255                 1260

Ile Asp  Ser Ile Lys Arg Val  Glu Ile Leu Ser Glu  Val Gln Ala
    1265                 1270                 1275

Met Leu  Asn Val Glu Ala Lys  Asp Val Asp Ala Leu  Ser Arg Thr
    1280                 1285                 1290

Arg Thr  Val Gly Glu Val Val  Asn Ala Met Lys Ala  Glu Ile Ala
    1295                 1300                 1305

Gly Gly  Ser Ala Pro Ala Pro  Ala Ala Ala Ala Pro  Gly Pro Ala
    1310                 1315                 1320

Ala Ala  Ala Pro Ala Pro Ala  Ala Ala Ala Pro Ala  Val Ser Asn
    1325                 1330                 1335

Glu Leu  Leu Glu Lys Ala Glu  Thr Val Val Met Glu  Val Leu Ala
    1340                 1345                 1350

Ala Lys  Thr Gly Tyr Glu Thr  Asp Met Ile Glu Ser  Asp Met Glu
    1355                 1360                 1365

Leu Glu  Thr Glu Leu Gly Ile  Asp Ser Ile Lys Arg  Val Glu Ile
    1370                 1375                 1380

Leu Ser  Glu Val Gln Ala Met  Leu Asn Val Glu Ala  Lys Asp Val
    1385                 1390                 1395

Asp Ala  Leu Ser Arg Thr Arg  Thr Val Gly Glu Val  Val Asp Ala
    1400                 1405                 1410

Met Lys  Ala Glu Ile Ala Gly  Gly Ser Ala Pro Ala  Pro Ala Ala
    1415                 1420                 1425

Ala Ala  Pro Ala Pro Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Pro
    1430                 1435                 1440

Ala Pro  Ala Val Ser Ser Glu  Leu Leu Glu Lys Ala  Glu Thr Val
    1445                 1450                 1455

Val Met  Glu Val Leu Ala Ala  Lys Thr Gly Tyr Glu  Thr Asp Met
    1460                 1465                 1470
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Ile Glu  Ser Asp Met Glu Leu  Glu Thr Glu Leu Gly  Ile Asp Ser
    1475                 1480                 1485

Ile Lys  Arg Val Glu Ile Leu  Ser Glu Val Gln Ala  Met Leu Asn
    1490                 1495                 1500

Val Glu  Ala Lys Asp Val Asp  Ala Leu Ser Arg Thr  Arg Thr Val
    1505                 1510                 1515

Gly Glu  Val Val Asp Ala Met  Lys Ala Glu Ile Ala  Gly Gly Ser
    1520                 1525                 1530

Ala Pro  Ala Pro Ala Ala Ala  Ala Pro Ala Pro Ala  Ala Ala Ala
    1535                 1540                 1545

Pro Ala  Pro Ala Ala Pro Ala  Pro Ala Ala Pro Ala  Pro Ala Val
    1550                 1555                 1560

Ser Ser  Glu Leu Leu Glu Lys  Ala Glu Thr Val Val  Met Glu Val
    1565                 1570                 1575

Leu Ala  Ala Lys Thr Gly Tyr  Glu Thr Asp Met Ile  Glu Ser Asp
    1580                 1585                 1590

Met Glu  Leu Glu Thr Glu Leu  Gly Ile Asp Ser Ile  Lys Arg Val
    1595                 1600                 1605

Glu Ile  Leu Ser Glu Val Gln  Ala Met Leu Asn Val  Glu Ala Lys
    1610                 1615                 1620

Asp Val  Asp Ala Leu Ser Arg  Thr Arg Thr Val Gly  Glu Val Val
    1625                 1630                 1635

Asp Ala  Met Lys Ala Glu Ile  Ala Gly Ser Ser Ala  Ser Ala Pro
    1640                 1645                 1650

Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Ala Ala Pro  Ala Pro Ala
    1655                 1660                 1665

Ala Ala  Ala Pro Ala Val Ser  Asn Glu Leu Leu Glu  Lys Ala Glu
    1670                 1675                 1680

Thr Val  Val Met Glu Val Leu  Ala Ala Lys Thr Gly  Tyr Glu Thr
    1685                 1690                 1695

Asp Met  Ile Glu Ser Asp Met  Glu Leu Glu Thr Glu  Leu Gly Ile
    1700                 1705                 1710

Asp Ser  Ile Lys Arg Val Glu  Ile Leu Ser Glu Val  Gln Ala Met
    1715                 1720                 1725

Leu Asn  Val Glu Ala Lys Asp  Val Asp Ala Leu Ser  Arg Thr Arg
    1730                 1735                 1740

Thr Val  Gly Glu Val Val Asp  Ala Met Lys Ala Glu  Ile Ala Gly
    1745                 1750                 1755

Gly Ser  Ala Pro Ala Pro Ala  Ala Ala Ala Pro Ala  Pro Ala Ala
    1760                 1765                 1770

Ala Ala  Pro Ala Val Ser Asn  Glu Leu Leu Glu Lys  Ala Glu Thr
    1775                 1780                 1785

Val Val  Met Glu Val Leu Ala  Ala Lys Thr Gly Tyr  Glu Thr Asp
    1790                 1795                 1800

Met Ile  Glu Ser Asp Met Glu  Leu Glu Thr Glu Leu  Gly Ile Asp
    1805                 1810                 1815

Ser Ile  Lys Arg Val Glu Ile  Leu Ser Glu Val Gln  Ala Met Leu
    1820                 1825                 1830

Asn Val  Glu Ala Lys Asp Val  Asp Ala Leu Ser Arg  Thr Arg Thr
    1835                 1840                 1845

Val Gly  Glu Val Val Asp Ala  Met Lys Ala Glu Ile  Ala Gly Ser
    1850                 1855                 1860

Ser Ala  Pro Ala Pro Ala Ala  Ala Ala Pro Ala Pro  Ala Ala Ala
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    1865                1870                1875

Ala Pro  Ala Pro Ala Ala Ala  Ala Pro Ala Val Ser  Ser Glu Leu
    1880                1885                1890

Leu Glu  Lys Ala Glu Thr Val  Val Met Glu Val Leu  Ala Ala Lys
    1895                1900                1905

Thr Gly  Tyr Glu Thr Asp Met  Ile Glu Ser Asp Met  Glu Leu Glu
    1910                1915                1920

Thr Glu  Leu Gly Ile Asp Ser  Ile Lys Arg Val Glu  Ile Leu Ser
    1925                1930                1935

Glu Val  Gln Ala Met Leu Asn  Val Glu Ala Lys Asp  Val Asp Ala
    1940                1945                1950

Leu Ser  Arg Thr Arg Thr Val  Gly Glu Val Val Asp  Ala Met Lys
    1955                1960                1965

Ala Glu  Ile Ala Gly Gly Ser  Ala Pro Ala Pro Ala  Ala Ala Ala
    1970                1975                1980

Pro Ala  Pro Ala Ala Ala Ala  Pro Ala Val Ser Asn  Glu Leu Leu
    1985                1990                1995

Glu Lys  Ala Glu Thr Val Val  Met Glu Val Leu Ala  Ala Lys Thr
    2000                2005                2010

Gly Tyr  Glu Thr Asp Met Ile  Glu Ser Asp Met Glu  Leu Glu Thr
    2015                2020                2025

Glu Leu  Gly Ile Asp Ser Ile  Lys Arg Val Glu Ile  Leu Ser Glu
    2030                2035                2040

Val Gln  Ala Met Leu Asn Val  Glu Ala Lys Asp Val  Asp Ala Leu
    2045                2050                2055

Ser Arg  Thr Arg Thr Val Gly  Glu Val Val Asp Ala  Met Lys Ala
    2060                2065                2070

Glu Ile  Ala Gly Gly Ser Ala  Pro Ala Pro Ala Ala  Ala Ala Pro
    2075                2080                2085

Ala Ser  Ala Gly Ala Ala Pro  Ala Val Lys Ile Asp  Ser Val His
    2090                2095                2100

Gly Ala  Asp Cys Asp Asp Leu  Ser Leu Met His Ala  Lys Val Val
    2105                2110                2115

Asp Ile  Arg Arg Pro Asp Glu  Leu Ile Leu Glu Arg  Pro Glu Asn
    2120                2125                2130

Arg Pro  Val Leu Val Val Asp  Asp Gly Ser Glu Leu  Thr Leu Ala
    2135                2140                2145

Leu Val  Arg Val Leu Gly Ala  Cys Ala Val Val Leu  Thr Phe Glu
    2150                2155                2160

Gly Leu  Gln Leu Ala Gln Arg  Ala Gly Ala Ala Ala  Ile Arg His
    2165                2170                2175

Val Leu  Ala Lys Asp Leu Ser  Ala Glu Ser Ala Glu  Lys Ala Ile
    2180                2185                2190

Lys Glu  Ala Glu Gln Arg Phe  Gly Ala Leu Gly Gly  Phe Ile Ser
    2195                2200                2205

Gln Gln  Ala Glu Arg Phe Glu  Pro Ala Glu Ile Leu  Gly Phe Thr
    2210                2215                2220

Leu Met  Cys Ala Lys Phe Ala  Lys Ala Ser Leu Cys  Thr Ala Val
    2225                2230                2235

Ala Gly  Gly Arg Pro Ala Phe  Ile Gly Val Ala Arg  Leu Asp Gly
    2240                2245                2250

Arg Leu  Gly Phe Thr Ser Gln  Gly Thr Ser Asp Ala  Leu Lys Arg
    2255                2260                2265
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Ala Gln  Arg Gly Ala Ile Phe  Gly Leu Cys Lys Thr  Ile Gly Leu
    2270                 2275                 2280

Glu Trp  Ser Glu Ser Asp Val  Phe Ser Arg Gly Val  Asp Ile Ala
    2285                 2290                 2295

Gln Gly  Met His Pro Glu Asp  Ala Ala Val Ala Ile  Val Arg Glu
    2300                 2305                 2310

Met Ala  Cys Ala Asp Ile Arg  Ile Arg Glu Val Gly  Ile Gly Ala
    2315                 2320                 2325

Asn Gln  Gln Arg Cys Thr Ile  Arg Ala Ala Lys Leu  Glu Thr Gly
    2330                 2335                 2340

Asn Pro  Gln Arg Gln Ile Ala  Lys Asp Asp Val Leu  Leu Val Ser
    2345                 2350                 2355

Gly Gly  Ala Arg Gly Ile Thr  Pro Leu Cys Ile Arg  Glu Ile Thr
    2360                 2365                 2370

Arg Gln  Ile Ala Gly Gly Lys  Tyr Ile Leu Leu Gly  Arg Ser Lys
    2375                 2380                 2385

Val Ser  Ala Ser Glu Pro Ala  Trp Cys Ala Gly Ile  Thr Asp Glu
    2390                 2395                 2400

Lys Ala  Val Gln Lys Ala Ala  Thr Gln Glu Leu Lys  Arg Ala Phe
    2405                 2410                 2415

Ser Ala  Gly Glu Gly Pro Lys  Pro Thr Pro Arg Ala  Val Thr Lys
    2420                 2425                 2430

Leu Val  Gly Ser Val Leu Gly  Ala Arg Glu Val Arg  Ser Ser Ile
    2435                 2440                 2445

Ala Ala  Ile Glu Ala Leu Gly  Gly Lys Ala Ile Tyr  Ser Ser Cys
    2450                 2455                 2460

Asp Val  Asn Ser Ala Ala Asp  Val Ala Lys Ala Val  Arg Asp Ala
    2465                 2470                 2475

Glu Ser  Gln Leu Gly Ala Arg  Val Ser Gly Ile Val  His Ala Ser
    2480                 2485                 2490

Gly Val  Leu Arg Asp Arg Leu  Ile Glu Lys Lys Leu  Pro Asp Glu
    2495                 2500                 2505

Phe Asp  Ala Val Phe Gly Thr  Lys Val Thr Gly Leu  Glu Asn Leu
    2510                 2515                 2520

Leu Ala  Ala Val Asp Arg Ala  Asn Leu Lys His Met  Val Leu Phe
    2525                 2530                 2535

Ser Ser  Leu Ala Gly Phe His  Gly Asn Val Gly Gln  Ser Asp Tyr
    2540                 2545                 2550

Ala Met  Ala Asn Glu Ala Leu  Asn Lys Met Gly Leu  Glu Leu Ala
    2555                 2560                 2565

Lys Asp  Val Ser Val Lys Ser  Ile Cys Phe Gly Pro  Trp Asp Gly
    2570                 2575                 2580

Gly Met  Val Thr Pro Gln Leu  Lys Lys Gln Phe Gln  Glu Met Gly
    2585                 2590                 2595

Val Gln  Ile Ile Pro Arg Glu  Gly Gly Ala Asp Thr  Val Ala Arg
    2600                 2605                 2610

Ile Val  Leu Gly Ser Ser Pro  Ala Glu Ile Leu Val  Gly Asn Trp
    2615                 2620                 2625

Arg Thr  Pro Ser Lys Lys Val  Gly Ser Asp Thr Ile  Thr Leu His
    2630                 2635                 2640

Arg Lys  Ile Ser Ala Lys Ser  Asn Pro Phe Leu Glu  Asp His Val
    2645                 2650                 2655
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Ile Gln  Gly Arg Arg Val Leu  Pro Met Thr Leu Ala  Ile Gly Ser
    2660                 2665                 2670

Leu Ala  Glu Thr Cys Leu Gly  Leu Phe Pro Gly Tyr  Ser Leu Trp
    2675                 2680                 2685

Ala Ile  Asp Asp Ala Gln Leu  Phe Lys Gly Val Thr  Val Asp Gly
    2690                 2695                 2700

Asp Val  Asn Cys Glu Val Thr  Leu Thr Pro Ser Thr  Ala Pro Ser
    2705                 2710                 2715

Gly Arg  Val Asn Val Gln Ala  Thr Leu Lys Thr Phe  Ser Ser Gly
    2720                 2725                 2730

Lys Leu  Val Pro Ala Tyr Arg  Ala Val Ile Val Leu  Ser Asn Gln
    2735                 2740                 2745

Gly Ala  Pro Pro Ala Asn Ala  Thr Met Gln Pro Pro  Ser Leu Asp
    2750                 2755                 2760

Ala Asp  Pro Ala Leu Gln Gly  Ser Val Tyr Asp Gly  Lys Thr Leu
    2765                 2770                 2775

Phe His  Gly Pro Ala Phe Arg  Gly Ile Asp Asp Val  Leu Ser Cys
    2780                 2785                 2790

Thr Lys  Ser Gln Leu Val Ala  Lys Cys Ser Ala Val  Pro Gly Ser
    2795                 2800                 2805

Asp Ala  Ala Arg Gly Glu Phe  Ala Thr Asp Thr Asp  Ala His Asp
    2810                 2815                 2820

Pro Phe  Val Asn Asp Leu Ala  Phe Gln Ala Met Leu  Val Trp Val
    2825                 2830                 2835

Arg Arg  Thr Leu Gly Gln Ala  Ala Leu Pro Asn Ser  Ile Gln Arg
    2840                 2845                 2850

Ile Val  Gln His Arg Pro Val  Pro Gln Asp Lys Pro  Phe Tyr Ile
    2855                 2860                 2865

Thr Leu  Arg Ser Asn Gln Ser  Gly Gly His Ser Gln  His Lys His
    2870                 2875                 2880

Ala Leu  Gln Phe His Asn Glu  Gln Gly Asp Leu Phe  Ile Asp Val
    2885                 2890                 2895

Gln Ala  Ser Val Ile Ala Thr  Asp Ser Leu Ala Phe
    2900                 2905                 2910


<210> SEQ ID NO 3
<211> LENGTH: 6177
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(6177)

<400> SEQUENCE: 3

atg gcc gct cgg aat gtg agc gcc gcg cat gag atg cac gat gaa aag       48
Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

cgc atc gcc gtc gtc ggc atg gcc gtc cag tac gcc gga tgc aaa acc       96
Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

aag gac gag ttc tgg gag gtg ctc atg aac ggc aag gtc gag tcc aag      144
Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

gtg atc agc gac aaa cga ctc ggc tcc aac tac cgc gcc gag cac tac      192
Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

aaa gca gag cgc agc aag tat gcc gac acc ttt tgc aac gaa acg tac      240
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Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80

ggc acc ctt gac gag aac gag atc gac aac gag cac gaa ctc ctc ctc      288
Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95

aac ctc gcc aag cag gca ctc gca gag aca tcc gtc aaa gac tcg aca      336
Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

cgc tgc ggc atc gtc agc ggc tgc ctc tcg ttc ccc atg gac aac ctc      384
Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

cag ggt gaa ctc ctc aac gtg tac caa aac cat gtc gag aaa aag ctc      432
Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

ggg gcc cgc gtc ttc aag gac gcc tcc cat tgg tcc gaa cgc gag cag      480
Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

tcc aac aaa ccc gag gcc ggt gac cgc cgc atc ttc atg gac ccg gcc      528
Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

tcc ttc gtc gcc gaa gaa ctc aac ctc ggc gcc ctt cac tac tcc gtc      576
Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

gac gca gca tgc gcc acg gcg ctc tac gtg ctc cgc ctc gcg cag gat      624
Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205

cat ctc gtc tcc ggc gcc gcc gac gtc atg ctc tgc ggt gcc acc tgc      672
His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

ctg ccg gag ccc ttt ttc atc ctt tcg ggc ttt tcc acc ttc cag gcc      720
Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

atg ccc gtc ggc acg ggc cag aac gtg tcc atg ccg ctg cac aag gac      768
Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

agc cag ggc ctc acc ccg ggt gag ggc ggc tcc atc atg gtc ctc aag      816
Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270

cgt ctc gat gat gcc atc cgc gac ggc gac cac att tac ggc acc ctt      864
Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

ctc ggc gcc aat gtc agc aac tcc ggc aca ggt ctg ccc ctc aag ccc      912
Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

ctt ctc ccc agc gag aaa aag tgc ctc atg gac acc tac acg cgc att      960
Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

aac gtg cac ccg cac aag att cag tac gtc gag tgc cac gcc acc ggc     1008
Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

acg ccc cag ggt gat cgt gtg gaa atc gac gcc gtc aag gcc tgc ttt     1056
Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

gaa ggc aag gtc ccc cgt ttc ggt acc aca aag ggc aac ttt gga cac     1104
Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

acc cts gyc gca gcc ggc ttt gcc ggt atg tgc aag gtc ctc ctc tcc     1152
Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380
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atg aag cat ggc atc atc ccg ccc acc ccg ggt atc gat gac gag acc      1200
Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

aag atg gac cct ctc gtc gtc tcc ggt gag gcc atc cca tgg cca gag      1248
Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415

acc aac ggc gag ccc aag cgc gcc ggt ctc tcg gcc ttt ggc ttt ggt      1296
Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

ggc acc aac gcc cat gcc gtc ttt gag gag cat gac ccc tcc aac gcc      1344
Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

gcc tgc acg ggc cac gac tcc att tct gcg ctc tcg gcc cgc tgc ggc      1392
Ala Cys Thr Gly His Asp Ser Ile Ser Ala Leu Ser Ala Arg Cys Gly
    450                 455                 460

ggt gaa agc aac atg cgc atc gcc atc act ggt atg gac gcc acc ttt      1440
Gly Glu Ser Asn Met Arg Ile Ala Ile Thr Gly Met Asp Ala Thr Phe
465                 470                 475                 480

ggc gct ctc aag gga ctc gac gcc ttc gag cgc gcc att tac acc ggc      1488
Gly Ala Leu Lys Gly Leu Asp Ala Phe Glu Arg Ala Ile Tyr Thr Gly
                485                 490                 495

gct cac ggt gcc atc cca ctc cca gaa aag cgc tgg cgc ttt ctc ggc      1536
Ala His Gly Ala Ile Pro Leu Pro Glu Lys Arg Trp Arg Phe Leu Gly
            500                 505                 510

aag gac aag gac ttt ctt gac ctc tgc ggc gtc aag gcc acc ccg cac      1584
Lys Asp Lys Asp Phe Leu Asp Leu Cys Gly Val Lys Ala Thr Pro His
        515                 520                 525

ggc tgc tac att gaa gat gtt gag gtc gac ttc cag cgc ctc cgc acg      1632
Gly Cys Tyr Ile Glu Asp Val Glu Val Asp Phe Gln Arg Leu Arg Thr
    530                 535                 540

ccc atg acc cct gaa gac atg ctc ctc cct cag cag ctt ctg gcc gtc      1680
Pro Met Thr Pro Glu Asp Met Leu Leu Pro Gln Gln Leu Leu Ala Val
545                 550                 555                 560

acc acc att gac cgc gcc atc ctc gac tcg gga atg aaa aag ggt ggc      1728
Thr Thr Ile Asp Arg Ala Ile Leu Asp Ser Gly Met Lys Lys Gly Gly
                565                 570                 575

aat gtc gcc gtc ttt gtc ggc ctc ggc acc gac ctc gag ctc tac cgt      1776
Asn Val Ala Val Phe Val Gly Leu Gly Thr Asp Leu Glu Leu Tyr Arg
            580                 585                 590

cac cgt gct cgc gtc gct ctc aag gag cgc gtc cgc cct gaa gcc tcc      1824
His Arg Ala Arg Val Ala Leu Lys Glu Arg Val Arg Pro Glu Ala Ser
        595                 600                 605

aag aag ctc aat gac atg atg cag tac att aac gac tgc ggc aca tcc      1872
Lys Lys Leu Asn Asp Met Met Gln Tyr Ile Asn Asp Cys Gly Thr Ser
    610                 615                 620

aca tcg tac acc tcg tac att ggc aac ctc gtc gcc acg cgc gtc tcg      1920
Thr Ser Tyr Thr Ser Tyr Ile Gly Asn Leu Val Ala Thr Arg Val Ser
625                 630                 635                 640

tcg cag tgg ggc ttc acg ggc ccc tcc ttt acg atc acc gag ggc aac      1968
Ser Gln Trp Gly Phe Thr Gly Pro Ser Phe Thr Ile Thr Glu Gly Asn
                645                 650                 655

aac tcc gtc tac cgc tgc gcc gag ctc ggc aag tac ctc ctc gag acc      2016
Asn Ser Val Tyr Arg Cys Ala Glu Leu Gly Lys Tyr Leu Leu Glu Thr
            660                 665                 670

ggc gag gtc gat ggc gtc gtc gtt gcg ggt gtc gat ctc tgc ggc agt      2064
Gly Glu Val Asp Gly Val Val Val Ala Gly Val Asp Leu Cys Gly Ser
        675                 680                 685

gcc gaa aac ctt tac gtc aag tct cgc cgc ttc aag gtg tcc acc tcc      2112
Ala Glu Asn Leu Tyr Val Lys Ser Arg Arg Phe Lys Val Ser Thr Ser
    690                 695                 700
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gat acc ccg cgc gcc agc ttt gac gcc gcc gcc gat ggc tac ttt gtc      2160
Asp Thr Pro Arg Ala Ser Phe Asp Ala Ala Ala Asp Gly Tyr Phe Val
705                 710                 715                 720

ggc gag ggc tgc ggt gcc ttt gtg ctc aag cgt gag act agc tgc acc      2208
Gly Glu Gly Cys Gly Ala Phe Val Leu Lys Arg Glu Thr Ser Cys Thr
                725                 730                 735

aag gac gac cgt atc tac gct tgc atg gat gcc atc gtc cct ggc aac      2256
Lys Asp Asp Arg Ile Tyr Ala Cys Met Asp Ala Ile Val Pro Gly Asn
            740                 745                 750

gtc cct agc gcc tgc ttg cgc gag gcc ctc gac cag gcg cgc gtc aag      2304
Val Pro Ser Ala Cys Leu Arg Glu Ala Leu Asp Gln Ala Arg Val Lys
        755                 760                 765

ccg ggc gat atc gag atg ctc gag ctc agc gcc gac tcc gcc cgc cac      2352
Pro Gly Asp Ile Glu Met Leu Glu Leu Ser Ala Asp Ser Ala Arg His
    770                 775                 780

ctc aag gac ccg tcc gtc ctg ccc aag gag ctc act gcc gag gag gaa      2400
Leu Lys Asp Pro Ser Val Leu Pro Lys Glu Leu Thr Ala Glu Glu Glu
785                 790                 795                 800

atc ggc ggc ctt cag acg atc ctt cgt gac gat gac aag ctc ccg cgc      2448
Ile Gly Gly Leu Gln Thr Ile Leu Arg Asp Asp Asp Lys Leu Pro Arg
                805                 810                 815

aac gtc gca acg ggc agt gtc aag gcc acc gtc ggt gac acc ggt tat      2496
Asn Val Ala Thr Gly Ser Val Lys Ala Thr Val Gly Asp Thr Gly Tyr
            820                 825                 830

gcc tct ggt gct gcc agc ctc atc aag gct gcg ctt tgc atc tac aac      2544
Ala Ser Gly Ala Ala Ser Leu Ile Lys Ala Ala Leu Cys Ile Tyr Asn
        835                 840                 845

cgc tac ctg ccc agc aac ggc gac gac tgg gat gaa ccc gcc cct gag      2592
Arg Tyr Leu Pro Ser Asn Gly Asp Asp Trp Asp Glu Pro Ala Pro Glu
    850                 855                 860

gcg ccc tgg gac agc acc ctc ttt gcg tgc cag acc tcg cgc gct tgg      2640
Ala Pro Trp Asp Ser Thr Leu Phe Ala Cys Gln Thr Ser Arg Ala Trp
865                 870                 875                 880

ctc aag aac cct ggc gag cgt cgc tat gcg gcc gtc tcg ggc gtc tcc      2688
Leu Lys Asn Pro Gly Glu Arg Arg Tyr Ala Ala Val Ser Gly Val Ser
                885                 890                 895

gag acg cgc tcg tgc tat tcc gtg ctc ctc tcc gaa gcc gag ggc cac      2736
Glu Thr Arg Ser Cys Tyr Ser Val Leu Leu Ser Glu Ala Glu Gly His
            900                 905                 910

tac gag cgc gag aac cgc atc tcg ctc gac gag gag gcg ccc aag ctc      2784
Tyr Glu Arg Glu Asn Arg Ile Ser Leu Asp Glu Glu Ala Pro Lys Leu
        915                 920                 925

att gtg ctt cgc gcc gac tcc cac gag gag atc ctt ggt cgc ctc gac      2832
Ile Val Leu Arg Ala Asp Ser His Glu Glu Ile Leu Gly Arg Leu Asp
    930                 935                 940

aag atc cgc gag cgc ttc ttg cag ccc acg ggc gcc gcc ccg cgc gag      2880
Lys Ile Arg Glu Arg Phe Leu Gln Pro Thr Gly Ala Ala Pro Arg Glu
945                 950                 955                 960

tcc gag ctc aag gcg cag gcc cgc cgc atc ttc ctc gag ctc ctc ggc      2928
Ser Glu Leu Lys Ala Gln Ala Arg Arg Ile Phe Leu Glu Leu Leu Gly
                965                 970                 975

gag acc ctt gcc cag gat gcc gct tct tca ggc tcg caa aag ccc ctc      2976
Glu Thr Leu Ala Gln Asp Ala Ala Ser Ser Gly Ser Gln Lys Pro Leu
            980                 985                 990

gct ctc agc ctc gtc tcc acg ccc  tcc aag ctc cag cgc  gag gtc gag    3024
Ala Leu Ser Leu Val Ser Thr Pro  Ser Lys Leu Gln Arg  Glu Val Glu
        995                 1000                 1005

ctc gcg  gcc aag ggt atc ccg  cgc tgc ctc aag atg  cgc cgc gat      3069
Leu Ala  Ala Lys Gly Ile Pro  Arg Cys Leu Lys Met  Arg Arg Asp
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    1010                1015                1020

tgg agc  tcc cct gct ggc agc  cgc tac gcg cct gag  ccg ctc gcc      3114
Trp Ser  Ser Pro Ala Gly Ser  Arg Tyr Ala Pro Glu  Pro Leu Ala
    1025                1030                1035

agc gac  cgc gtc gcc ttc atg  tac ggc gaa ggt cgc  agc cct tac      3159
Ser Asp  Arg Val Ala Phe Met  Tyr Gly Glu Gly Arg  Ser Pro Tyr
    1040                1045                1050

tac ggc  atc acc caa gac att  cac cgc att tgg ccc  gaa ctc cac      3204
Tyr Gly  Ile Thr Gln Asp Ile  His Arg Ile Trp Pro  Glu Leu His
    1055                1060                1065

gag gtc  atc aac gaa aag acg  aac cgt ctc tgg gcc  gaa ggc gac      3249
Glu Val  Ile Asn Glu Lys Thr  Asn Arg Leu Trp Ala  Glu Gly Asp
    1070                1075                1080

cgc tgg  gtc atg ccg cgc gcc  agc ttc aag tcg gag  ctc gag agc      3294
Arg Trp  Val Met Pro Arg Ala  Ser Phe Lys Ser Glu  Leu Glu Ser
    1085                1090                1095

cag cag  caa gag ttt gat cgc  aac atg att gaa atg  ttc cgt ctt      3339
Gln Gln  Gln Glu Phe Asp Arg  Asn Met Ile Glu Met  Phe Arg Leu
    1100                1105                1110

gga atc  ctc acc tca att gcc  ttc acc aat ctg gcg  cgc gac gtt      3384
Gly Ile  Leu Thr Ser Ile Ala  Phe Thr Asn Leu Ala  Arg Asp Val
    1115                1120                1125

ctc aac  atc acg ccc aag gcc  gcc ttt ggc ctc agt  ctt ggc gag      3429
Leu Asn  Ile Thr Pro Lys Ala  Ala Phe Gly Leu Ser  Leu Gly Glu
    1130                1135                1140

att tcc  atg att ttt gcc ttt  tcc aag aag aac ggt  ctc atc tcc      3474
Ile Ser  Met Ile Phe Ala Phe  Ser Lys Lys Asn Gly  Leu Ile Ser
    1145                1150                1155

gac cag  ctc acc aag gat ctt  cgc gag tcc gac gtg  tgg aac aag      3519
Asp Gln  Leu Thr Lys Asp Leu  Arg Glu Ser Asp Val  Trp Asn Lys
    1160                1165                1170

gct ctg  gcc gtt gaa ttt aat  gcg ctg cgc gag gcc  tgg ggc att      3564
Ala Leu  Ala Val Glu Phe Asn  Ala Leu Arg Glu Ala  Trp Gly Ile
    1175                1180                1185

cca cag  agt gtc ccc aag gac  gag ttc tgg caa ggc  tac att gtg      3609
Pro Gln  Ser Val Pro Lys Asp  Glu Phe Trp Gln Gly  Tyr Ile Val
    1190                1195                1200

cgc ggc  acc aag cag gat atc  gag gcg gcc atc gcc  ccg gac agc      3654
Arg Gly  Thr Lys Gln Asp Ile  Glu Ala Ala Ile Ala  Pro Asp Ser
    1205                1210                1215

aag tac  gtg cgc ctc acc atc  atc aat gat gcc aac  acc gcc ctc      3699
Lys Tyr  Val Arg Leu Thr Ile  Ile Asn Asp Ala Asn  Thr Ala Leu
    1220                1225                1230

att agc  ggc aag ccc gac gcc  tgc aag gct gcg atc  gcg cgt ctc      3744
Ile Ser  Gly Lys Pro Asp Ala  Cys Lys Ala Ala Ile  Ala Arg Leu
    1235                1240                1245

ggt ggc  aac att cct gcg ctt  ccc gtg acc cag ggc  atg tgc ggc      3789
Gly Gly  Asn Ile Pro Ala Leu  Pro Val Thr Gln Gly  Met Cys Gly
    1250                1255                1260

cac tgc  ccc gag gtg gga cct  tat acc aag gat atc  gcc aag atc      3834
His Cys  Pro Glu Val Gly Pro  Tyr Thr Lys Asp Ile  Ala Lys Ile
    1265                1270                1275

cat gcc  aac ctt gag ttc ccc  gtt gtc gac ggc ctt  gac ctc tgg      3879
His Ala  Asn Leu Glu Phe Pro  Val Val Asp Gly Leu  Asp Leu Trp
    1280                1285                1290

acc aca  atc aac cag aag cgc  ctc gtg cca cgc gcc  acg ggc gcc      3924
Thr Thr  Ile Asn Gln Lys Arg  Leu Val Pro Arg Ala  Thr Gly Ala
    1295                1300                1305

aag gac  gaa tgg gcc cct tct  tcc ttt ggc gag tac  gcc ggc cag      3969
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Lys Asp  Glu Trp Ala Pro Ser  Ser Phe Gly Glu Tyr  Ala Gly Gln
    1310                 1315                 1320

ctc tac  gag aag cag gct aac  ttc ccc caa atc gtc  gag acc att      4014
Leu Tyr  Glu Lys Gln Ala Asn  Phe Pro Gln Ile Val  Glu Thr Ile
    1325                 1330                 1335

tac aag  caa aac tac gac gtc  ttt gtc gag gtt ggg  ccc aac aac      4059
Tyr Lys  Gln Asn Tyr Asp Val  Phe Val Glu Val Gly  Pro Asn Asn
    1340                 1345                 1350

cac cgt  agc acc gca gtg cgc  acc acg ctt ggt ccc  cag cgc aac      4104
His Arg  Ser Thr Ala Val Arg  Thr Thr Leu Gly Pro  Gln Arg Asn
    1355                 1360                 1365

cac ctt  gct ggc gcc atc gac  aag cag aac gag gat  gct tgg acg      4149
His Leu  Ala Gly Ala Ile Asp  Lys Gln Asn Glu Asp  Ala Trp Thr
    1370                 1375                 1380

acc atc  gtc aag ctt gtg gct  tcg ctc aag gcc cac  ctt gtt cct      4194
Thr Ile  Val Lys Leu Val Ala  Ser Leu Lys Ala His  Leu Val Pro
    1385                 1390                 1395

ggc gtc  acg atc tcg ccg ctg  tac cac tcc aag ctt  gtg gcg gag      4239
Gly Val  Thr Ile Ser Pro Leu  Tyr His Ser Lys Leu  Val Ala Glu
    1400                 1405                 1410

gct cag  gct tgc tac gct gcg  ctc tgc aag ggt gaa  aag ccc aag      4284
Ala Gln  Ala Cys Tyr Ala Ala  Leu Cys Lys Gly Glu  Lys Pro Lys
    1415                 1420                 1425

aag aac  aag ttt gtg cgc aag  att cag ctc aac ggt  cgc ttc aac      4329
Lys Asn  Lys Phe Val Arg Lys  Ile Gln Leu Asn Gly  Arg Phe Asn
    1430                 1435                 1440

agc aag  gcg gac ccc atc tcc  tcg gcc gat ctt gcc  agc ttt ccg      4374
Ser Lys  Ala Asp Pro Ile Ser  Ser Ala Asp Leu Ala  Ser Phe Pro
    1445                 1450                 1455

cct gcg  gac cct gcc att gaa  gcc gcc atc tcg agc  cgc atc atg      4419
Pro Ala  Asp Pro Ala Ile Glu  Ala Ala Ile Ser Ser  Arg Ile Met
    1460                 1465                 1470

aag cct  gtc gct ccc aag ttc  tac gcg cgt ctc aac  att gac gag      4464
Lys Pro  Val Ala Pro Lys Phe  Tyr Ala Arg Leu Asn  Ile Asp Glu
    1475                 1480                 1485

cag gac  gag acc cga gat ccg  atc ctc aac aag gac  aac gcg ccg      4509
Gln Asp  Glu Thr Arg Asp Pro  Ile Leu Asn Lys Asp  Asn Ala Pro
    1490                 1495                 1500

tct tct  tct tct tct tct tct  tct tct tct tct tct  tct tct tct      4554
Ser Ser  Ser Ser Ser Ser Ser  Ser Ser Ser Ser Ser  Ser Ser Ser
    1505                 1510                 1515

ccg tcg  cct gct cct tcg gcc  ccc gtg caa aag aag  gct gct ccc      4599
Pro Ser  Pro Ala Pro Ser Ala  Pro Val Gln Lys Lys  Ala Ala Pro
    1520                 1525                 1530

gcc gcg  gag acc aag gct gtt  gct tcg gct gac gca  ctt cgc agt      4644
Ala Ala  Glu Thr Lys Ala Val  Ala Ser Ala Asp Ala  Leu Arg Ser
    1535                 1540                 1545

gcc ctg  ctc gat ctc gac agt  atg ctt gcg ctg agc  tct gcc agt      4689
Ala Leu  Leu Asp Leu Asp Ser  Met Leu Ala Leu Ser  Ser Ala Ser
    1550                 1555                 1560

gcc tcc  ggc aac ctt gtt gag  act gcg cct agc gac  gcc tcg gtc      4734
Ala Ser  Gly Asn Leu Val Glu  Thr Ala Pro Ser Asp  Ala Ser Val
    1565                 1570                 1575

att gtg  ccg ccc tgc aac att  gcg gat ctc ggc agc  cgc gcc ttc      4779
Ile Val  Pro Pro Cys Asn Ile  Ala Asp Leu Gly Ser  Arg Ala Phe
    1580                 1585                 1590

atg aaa  acg tac ggt gtt tcg  gcg cct ctg tac acg  ggc gcc atg      4824
Met Lys  Thr Tyr Gly Val Ser  Ala Pro Leu Tyr Thr  Gly Ala Met
    1595                 1600                 1605
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gcc aag  ggc att gcc tct gcg  gac ctc gtc att gcc  gcc ggc cgc      4869
Ala Lys  Gly Ile Ala Ser Ala  Asp Leu Val Ile Ala  Ala Gly Arg
    1610                 1615                 1620

cag ggc  atc ctt gcg tcc ttt  ggc gcc ggc gga ctt  ccc atg cag      4914
Gln Gly  Ile Leu Ala Ser Phe  Gly Ala Gly Gly Leu  Pro Met Gln
    1625                 1630                 1635

gtt gtg  cgt gag tcc atc gaa  aag att cag gcc gcc  ctg ccc aat      4959
Val Val  Arg Glu Ser Ile Glu  Lys Ile Gln Ala Ala  Leu Pro Asn
    1640                 1645                 1650

ggc ccg  tac gct gtc aac ctt  atc cat tct ccc ttt  gac agc aac      5004
Gly Pro  Tyr Ala Val Asn Leu  Ile His Ser Pro Phe  Asp Ser Asn
    1655                 1660                 1665

ctc gaa  aag ggc aat gtc gat  ctc ttc ctc gag aag  ggt gtc acc      5049
Leu Glu  Lys Gly Asn Val Asp  Leu Phe Leu Glu Lys  Gly Val Thr
    1670                 1675                 1680

ttt gtc  gag gcc tcg gcc ttt  atg acg ctc acc ccg  cag gtc gtg      5094
Phe Val  Glu Ala Ser Ala Phe  Met Thr Leu Thr Pro  Gln Val Val
    1685                 1690                 1695

cgg tac  cgc gcg gct ggc ctc  acg cgc aac gcc gac  ggc tcg gtc      5139
Arg Tyr  Arg Ala Ala Gly Leu  Thr Arg Asn Ala Asp  Gly Ser Val
    1700                 1705                 1710

aac atc  cgc aac cgt atc att  ggc aag gtc tcg cgc  acc gag ctc      5184
Asn Ile  Arg Asn Arg Ile Ile  Gly Lys Val Ser Arg  Thr Glu Leu
    1715                 1720                 1725

gcc gag  atg ttc atg cgt cct  gcg ccc gag cac ctt  ctt cag aag      5229
Ala Glu  Met Phe Met Arg Pro  Ala Pro Glu His Leu  Leu Gln Lys
    1730                 1735                 1740

ctc att  gct tcc ggc gag atc  aac cag gag cag gcc  gag ctc gcc      5274
Leu Ile  Ala Ser Gly Glu Ile  Asn Gln Glu Gln Ala  Glu Leu Ala
    1745                 1750                 1755

cgc cgt  gtt ccc gtc gct gac  gac atc gcg gtc gaa  gct gac tcg      5319
Arg Arg  Val Pro Val Ala Asp  Asp Ile Ala Val Glu  Ala Asp Ser
    1760                 1765                 1770

ggt ggc  cac acc gac aac cgc  ccc atc cac gtc att  ctg ccc ctc      5364
Gly Gly  His Thr Asp Asn Arg  Pro Ile His Val Ile  Leu Pro Leu
    1775                 1780                 1785

atc atc  aac ctt cgc gac cgc  ctt cac cgc gag tgc  ggc tac ccg      5409
Ile Ile  Asn Leu Arg Asp Arg  Leu His Arg Glu Cys  Gly Tyr Pro
    1790                 1795                 1800

gcc aac  ctt cgc gtc cgt gtg  ggc gcc ggc ggt ggc  att ggg tgc      5454
Ala Asn  Leu Arg Val Arg Val  Gly Ala Gly Gly Gly  Ile Gly Cys
    1805                 1810                 1815

ccc cag  gcg gcg ctg gcc acc  ttc aac atg ggt gcc  tcc ttt att      5499
Pro Gln  Ala Ala Leu Ala Thr  Phe Asn Met Gly Ala  Ser Phe Ile
    1820                 1825                 1830

gtc acc  ggc acc gtg aac cag  gtc gcc aag cag tcg  ggc acg tgc      5544
Val Thr  Gly Thr Val Asn Gln  Val Ala Lys Gln Ser  Gly Thr Cys
    1835                 1840                 1845

gac aat  gtg cgc aag cag ctc  gcg aag gcc act tac  tcg gac gta      5589
Asp Asn  Val Arg Lys Gln Leu  Ala Lys Ala Thr Tyr  Ser Asp Val
    1850                 1855                 1860

tgc atg  gcc ccg gct gcc gac  atg ttc gag gaa ggc  gtc aag ctt      5634
Cys Met  Ala Pro Ala Ala Asp  Met Phe Glu Glu Gly  Val Lys Leu
    1865                 1870                 1875

cag gtc  ctc aag aag gga acc  atg ttt ccc tcg cgc  gcc aac aag      5679
Gln Val  Leu Lys Lys Gly Thr  Met Phe Pro Ser Arg  Ala Asn Lys
    1880                 1885                 1890

ctc tac  gag ctc ttt tgc aag  tac gac tcg ttc gag  tcc atg ccc      5724
Leu Tyr  Glu Leu Phe Cys Lys  Tyr Asp Ser Phe Glu  Ser Met Pro
    1895                 1900                 1905
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ccc gca  gag ctt gcg cgc gtc  gag aag cgc atc ttc  agc cgc gcg      5769
Pro Ala  Glu Leu Ala Arg Val  Glu Lys Arg Ile Phe  Ser Arg Ala
    1910                 1915                 1920

ctc gaa  gag gtc tgg gac gag  acc aaa aac ttt tac  att aac cgt      5814
Leu Glu  Glu Val Trp Asp Glu  Thr Lys Asn Phe Tyr  Ile Asn Arg
    1925                 1930                 1935

ctt cac  aac ccg gag aag atc  cag cgc gcc gag cgc  gac ccc aag      5859
Leu His  Asn Pro Glu Lys Ile  Gln Arg Ala Glu Arg  Asp Pro Lys
    1940                 1945                 1950

ctc aag  atg tcg ctg tgc ttt  cgc tgg tac ctg agc  ctg gcg agc      5904
Leu Lys  Met Ser Leu Cys Phe  Arg Trp Tyr Leu Ser  Leu Ala Ser
    1955                 1960                 1965

cgc tgg  gcc aac act gga gct  tcc gat cgc gtc atg  gac tac cag      5949
Arg Trp  Ala Asn Thr Gly Ala  Ser Asp Arg Val Met  Asp Tyr Gln
    1970                 1975                 1980

gtc tgg  tgc ggt cct gcc att  ggt tcc ttc aac gat  ttc atc aag      5994
Val Trp  Cys Gly Pro Ala Ile  Gly Ser Phe Asn Asp  Phe Ile Lys
    1985                 1990                 1995

gga act  tac ctt gat ccg gcc  gtc gca aac gag tac  ccg tgc gtc      6039
Gly Thr  Tyr Leu Asp Pro Ala  Val Ala Asn Glu Tyr  Pro Cys Val
    2000                 2005                 2010

gtt cag  att aac aag cag atc  ctt cgt gga gcg tgc  ttc ttg cgc      6084
Val Gln  Ile Asn Lys Gln Ile  Leu Arg Gly Ala Cys  Phe Leu Arg
    2015                 2020                 2025

cgt ctc  gaa att ctg cgc aac  gca cgc ctt tcc gat  ggc gct gcc      6129
Arg Leu  Glu Ile Leu Arg Asn  Ala Arg Leu Ser Asp  Gly Ala Ala
    2030                 2035                 2040

gct ctt  gtg gcc agc atc gat  gac aca tac gtc ccg  gcc gag aag      6174
Ala Leu  Val Ala Ser Ile Asp  Asp Thr Tyr Val Pro  Ala Glu Lys
    2045                 2050                 2055

ctg                                                                 6177
Leu


<210> SEQ ID NO 4
<211> LENGTH: 2059
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (370)..(370)
<223> OTHER INFORMATION: The 'Xaa' at location 370 stands for Leu.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (371)..(371)
<223> OTHER INFORMATION: The 'Xaa' at location 371 stands for Ala, or
      Val.

<400> SEQUENCE: 4

Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80

Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95
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Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205

His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270

Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380

Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415

Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

Ala Cys Thr Gly His Asp Ser Ile Ser Ala Leu Ser Ala Arg Cys Gly
    450                 455                 460

Gly Glu Ser Asn Met Arg Ile Ala Ile Thr Gly Met Asp Ala Thr Phe
465                 470                 475                 480

Gly Ala Leu Lys Gly Leu Asp Ala Phe Glu Arg Ala Ile Tyr Thr Gly
                485                 490                 495

Ala His Gly Ala Ile Pro Leu Pro Glu Lys Arg Trp Arg Phe Leu Gly
            500                 505                 510

Lys Asp Lys Asp Phe Leu Asp Leu Cys Gly Val Lys Ala Thr Pro His
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        515                 520                 525

Gly Cys Tyr Ile Glu Asp Val Glu Val Asp Phe Gln Arg Leu Arg Thr
    530                 535                 540

Pro Met Thr Pro Glu Asp Met Leu Leu Pro Gln Gln Leu Leu Ala Val
545                 550                 555                 560

Thr Thr Ile Asp Arg Ala Ile Leu Asp Ser Gly Met Lys Lys Gly Gly
                565                 570                 575

Asn Val Ala Val Phe Val Gly Leu Gly Thr Asp Leu Glu Leu Tyr Arg
            580                 585                 590

His Arg Ala Arg Val Ala Leu Lys Glu Arg Val Arg Pro Glu Ala Ser
        595                 600                 605

Lys Lys Leu Asn Asp Met Met Gln Tyr Ile Asn Asp Cys Gly Thr Ser
    610                 615                 620

Thr Ser Tyr Thr Ser Tyr Ile Gly Asn Leu Val Ala Thr Arg Val Ser
625                 630                 635                 640

Ser Gln Trp Gly Phe Thr Gly Pro Ser Phe Thr Ile Thr Glu Gly Asn
                645                 650                 655

Asn Ser Val Tyr Arg Cys Ala Glu Leu Gly Lys Tyr Leu Leu Glu Thr
            660                 665                 670

Gly Glu Val Asp Gly Val Val Val Ala Gly Val Asp Leu Cys Gly Ser
        675                 680                 685

Ala Glu Asn Leu Tyr Val Lys Ser Arg Arg Phe Lys Val Ser Thr Ser
    690                 695                 700

Asp Thr Pro Arg Ala Ser Phe Asp Ala Ala Ala Asp Gly Tyr Phe Val
705                 710                 715                 720

Gly Glu Gly Cys Gly Ala Phe Val Leu Lys Arg Glu Thr Ser Cys Thr
                725                 730                 735

Lys Asp Asp Arg Ile Tyr Ala Cys Met Asp Ala Ile Val Pro Gly Asn
            740                 745                 750

Val Pro Ser Ala Cys Leu Arg Glu Ala Leu Asp Gln Ala Arg Val Lys
        755                 760                 765

Pro Gly Asp Ile Glu Met Leu Glu Leu Ser Ala Asp Ser Ala Arg His
    770                 775                 780

Leu Lys Asp Pro Ser Val Leu Pro Lys Glu Leu Thr Ala Glu Glu Glu
785                 790                 795                 800

Ile Gly Gly Leu Gln Thr Ile Leu Arg Asp Asp Asp Lys Leu Pro Arg
                805                 810                 815

Asn Val Ala Thr Gly Ser Val Lys Ala Thr Val Gly Asp Thr Gly Tyr
            820                 825                 830

Ala Ser Gly Ala Ala Ser Leu Ile Lys Ala Ala Leu Cys Ile Tyr Asn
        835                 840                 845

Arg Tyr Leu Pro Ser Asn Gly Asp Asp Trp Asp Glu Pro Ala Pro Glu
    850                 855                 860

Ala Pro Trp Asp Ser Thr Leu Phe Ala Cys Gln Thr Ser Arg Ala Trp
865                 870                 875                 880

Leu Lys Asn Pro Gly Glu Arg Arg Tyr Ala Ala Val Ser Gly Val Ser
                885                 890                 895

Glu Thr Arg Ser Cys Tyr Ser Val Leu Leu Ser Glu Ala Glu Gly His
            900                 905                 910

Tyr Glu Arg Glu Asn Arg Ile Ser Leu Asp Glu Glu Ala Pro Lys Leu
        915                 920                 925

Ile Val Leu Arg Ala Asp Ser His Glu Glu Ile Leu Gly Arg Leu Asp
    930                 935                 940
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Lys Ile Arg Glu Arg Phe Leu Gln Pro Thr Gly Ala Ala Pro Arg Glu
945                 950                 955                 960

Ser Glu Leu Lys Ala Gln Ala Arg Arg Ile Phe Leu Glu Leu Leu Gly
                965                 970                 975

Glu Thr Leu Ala Gln Asp Ala Ala Ser Ser Gly Ser Gln Lys Pro Leu
            980                 985                 990

Ala Leu Ser Leu Val Ser Thr Pro  Ser Lys Leu Gln Arg  Glu Val Glu
        995                 1000                1005

Leu Ala  Ala Lys Gly Ile Pro  Arg Cys Leu Lys Met  Arg Arg Asp
    1010                 1015                 1020

Trp Ser  Ser Pro Ala Gly Ser  Arg Tyr Ala Pro Glu  Pro Leu Ala
    1025                 1030                 1035

Ser Asp  Arg Val Ala Phe Met  Tyr Gly Glu Gly Arg  Ser Pro Tyr
    1040                 1045                 1050

Tyr Gly  Ile Thr Gln Asp Ile  His Arg Ile Trp Pro  Glu Leu His
    1055                 1060                 1065

Glu Val  Ile Asn Glu Lys Thr  Asn Arg Leu Trp Ala  Glu Gly Asp
    1070                 1075                 1080

Arg Trp  Val Met Pro Arg Ala  Ser Phe Lys Ser Glu  Leu Glu Ser
    1085                 1090                 1095

Gln Gln  Gln Glu Phe Asp Arg  Asn Met Ile Glu Met  Phe Arg Leu
    1100                 1105                 1110

Gly Ile  Leu Thr Ser Ile Ala  Phe Thr Asn Leu Ala  Arg Asp Val
    1115                 1120                 1125

Leu Asn  Ile Thr Pro Lys Ala  Ala Phe Gly Leu Ser  Leu Gly Glu
    1130                 1135                 1140

Ile Ser  Met Ile Phe Ala Phe  Ser Lys Lys Asn Gly  Leu Ile Ser
    1145                 1150                 1155

Asp Gln  Leu Thr Lys Asp Leu  Arg Glu Ser Asp Val  Trp Asn Lys
    1160                 1165                 1170

Ala Leu  Ala Val Glu Phe Asn  Ala Leu Arg Glu Ala  Trp Gly Ile
    1175                 1180                 1185

Pro Gln  Ser Val Pro Lys Asp  Glu Phe Trp Gln Gly  Tyr Ile Val
    1190                 1195                 1200

Arg Gly  Thr Lys Gln Asp Ile  Glu Ala Ala Ile Ala  Pro Asp Ser
    1205                 1210                 1215

Lys Tyr  Val Arg Leu Thr Ile  Ile Asn Asp Ala Asn  Thr Ala Leu
    1220                 1225                 1230

Ile Ser  Gly Lys Pro Asp Ala  Cys Lys Ala Ala Ile  Ala Arg Leu
    1235                 1240                 1245

Gly Gly  Asn Ile Pro Ala Leu  Pro Val Thr Gln Gly  Met Cys Gly
    1250                 1255                 1260

His Cys  Pro Glu Val Gly Pro  Tyr Thr Lys Asp Ile  Ala Lys Ile
    1265                 1270                 1275

His Ala  Asn Leu Glu Phe Pro  Val Val Asp Gly Leu  Asp Leu Trp
    1280                 1285                 1290

Thr Thr  Ile Asn Gln Lys Arg  Leu Val Pro Arg Ala  Thr Gly Ala
    1295                 1300                 1305

Lys Asp  Glu Trp Ala Pro Ser  Ser Phe Gly Glu Tyr  Ala Gly Gln
    1310                 1315                 1320

Leu Tyr  Glu Lys Gln Ala Asn  Phe Pro Gln Ile Val  Glu Thr Ile
    1325                 1330                 1335
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Tyr Lys  Gln Asn Tyr Asp Val  Phe Val Glu Val Gly  Pro Asn Asn
    1340                 1345                 1350

His Arg  Ser Thr Ala Val Arg  Thr Thr Leu Gly Pro  Gln Arg Asn
    1355                 1360                 1365

His Leu  Ala Gly Ala Ile Asp  Lys Gln Asn Glu Asp  Ala Trp Thr
    1370                 1375                 1380

Thr Ile  Val Lys Leu Val Ala  Ser Leu Lys Ala His  Leu Val Pro
    1385                 1390                 1395

Gly Val  Thr Ile Ser Pro Leu  Tyr His Ser Lys Leu  Val Ala Glu
    1400                 1405                 1410

Ala Gln  Ala Cys Tyr Ala Ala  Leu Cys Lys Gly Glu  Lys Pro Lys
    1415                 1420                 1425

Lys Asn  Lys Phe Val Arg Lys  Ile Gln Leu Asn Gly  Arg Phe Asn
    1430                 1435                 1440

Ser Lys  Ala Asp Pro Ile Ser  Ser Ala Asp Leu Ala  Ser Phe Pro
    1445                 1450                 1455

Pro Ala  Asp Pro Ala Ile Glu  Ala Ala Ile Ser Ser  Arg Ile Met
    1460                 1465                 1470

Lys Pro  Val Ala Pro Lys Phe  Tyr Ala Arg Leu Asn  Ile Asp Glu
    1475                 1480                 1485

Gln Asp  Glu Thr Arg Asp Pro  Ile Leu Asn Lys Asp  Asn Ala Pro
    1490                 1495                 1500

Ser Ser  Ser Ser Ser Ser Ser  Ser Ser Ser Ser Ser  Ser Ser Ser
    1505                 1510                 1515

Pro Ser  Pro Ala Pro Ser Ala  Pro Val Gln Lys Lys  Ala Ala Pro
    1520                 1525                 1530

Ala Ala  Glu Thr Lys Ala Val  Ala Ser Ala Asp Ala  Leu Arg Ser
    1535                 1540                 1545

Ala Leu  Leu Asp Leu Asp Ser  Met Leu Ala Leu Ser  Ser Ala Ser
    1550                 1555                 1560

Ala Ser  Gly Asn Leu Val Glu  Thr Ala Pro Ser Asp  Ala Ser Val
    1565                 1570                 1575

Ile Val  Pro Pro Cys Asn Ile  Ala Asp Leu Gly Ser  Arg Ala Phe
    1580                 1585                 1590

Met Lys  Thr Tyr Gly Val Ser  Ala Pro Leu Tyr Thr  Gly Ala Met
    1595                 1600                 1605

Ala Lys  Gly Ile Ala Ser Ala  Asp Leu Val Ile Ala  Ala Gly Arg
    1610                 1615                 1620

Gln Gly  Ile Leu Ala Ser Phe  Gly Ala Gly Gly Leu  Pro Met Gln
    1625                 1630                 1635

Val Val  Arg Glu Ser Ile Glu  Lys Ile Gln Ala Ala  Leu Pro Asn
    1640                 1645                 1650

Gly Pro  Tyr Ala Val Asn Leu  Ile His Ser Pro Phe  Asp Ser Asn
    1655                 1660                 1665

Leu Glu  Lys Gly Asn Val Asp  Leu Phe Leu Glu Lys  Gly Val Thr
    1670                 1675                 1680

Phe Val  Glu Ala Ser Ala Phe  Met Thr Leu Thr Pro  Gln Val Val
    1685                 1690                 1695

Arg Tyr  Arg Ala Ala Gly Leu  Thr Arg Asn Ala Asp  Gly Ser Val
    1700                 1705                 1710

Asn Ile  Arg Asn Arg Ile Ile  Gly Lys Val Ser Arg  Thr Glu Leu
    1715                 1720                 1725

Ala Glu  Met Phe Met Arg Pro  Ala Pro Glu His Leu  Leu Gln Lys
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    1730                1735                1740

Leu Ile  Ala Ser Gly Glu Ile  Asn Gln Glu Gln Ala  Glu Leu Ala
    1745                1750                1755

Arg Arg  Val Pro Val Ala Asp  Asp Ile Ala Val Glu  Ala Asp Ser
    1760                1765                1770

Gly Gly  His Thr Asp Asn Arg  Pro Ile His Val Ile  Leu Pro Leu
    1775                1780                1785

Ile Ile  Asn Leu Arg Asp Arg  Leu His Arg Glu Cys  Gly Tyr Pro
    1790                1795                1800

Ala Asn  Leu Arg Val Arg Val  Gly Ala Gly Gly Gly  Ile Gly Cys
    1805                1810                1815

Pro Gln  Ala Ala Leu Ala Thr  Phe Asn Met Gly Ala  Ser Phe Ile
    1820                1825                1830

Val Thr  Gly Thr Val Asn Gln  Val Ala Lys Gln Ser  Gly Thr Cys
    1835                1840                1845

Asp Asn  Val Arg Lys Gln Leu  Ala Lys Ala Thr Tyr  Ser Asp Val
    1850                1855                1860

Cys Met  Ala Pro Ala Ala Asp  Met Phe Glu Glu Gly  Val Lys Leu
    1865                1870                1875

Gln Val  Leu Lys Lys Gly Thr  Met Phe Pro Ser Arg  Ala Asn Lys
    1880                1885                1890

Leu Tyr  Glu Leu Phe Cys Lys  Tyr Asp Ser Phe Glu  Ser Met Pro
    1895                1900                1905

Pro Ala  Glu Leu Ala Arg Val  Glu Lys Arg Ile Phe  Ser Arg Ala
    1910                1915                1920

Leu Glu  Glu Val Trp Asp Glu  Thr Lys Asn Phe Tyr  Ile Asn Arg
    1925                1930                1935

Leu His  Asn Pro Glu Lys Ile  Gln Arg Ala Glu Arg  Asp Pro Lys
    1940                1945                1950

Leu Lys  Met Ser Leu Cys Phe  Arg Trp Tyr Leu Ser  Leu Ala Ser
    1955                1960                1965

Arg Trp  Ala Asn Thr Gly Ala  Ser Asp Arg Val Met  Asp Tyr Gln
    1970                1975                1980

Val Trp  Cys Gly Pro Ala Ile  Gly Ser Phe Asn Asp  Phe Ile Lys
    1985                1990                1995

Gly Thr  Tyr Leu Asp Pro Ala  Val Ala Asn Glu Tyr  Pro Cys Val
    2000                2005                2010

Val Gln  Ile Asn Lys Gln Ile  Leu Arg Gly Ala Cys  Phe Leu Arg
    2015                2020                2025

Arg Leu  Glu Ile Leu Arg Asn  Ala Arg Leu Ser Asp  Gly Ala Ala
    2030                2035                2040

Ala Leu  Val Ala Ser Ile Asp  Asp Thr Tyr Val Pro  Ala Glu Lys
    2045                2050                2055

Leu


<210> SEQ ID NO 5
<211> LENGTH: 4509
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(4509)

<400> SEQUENCE: 5

atg gcg ctc cgt gtc aag acg aac aag aag cca tgc tgg gag atg acc       48
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Met Ala Leu Arg Val Lys Thr Asn Lys Lys Pro Cys Trp Glu Met Thr
1               5                   10                  15

aag gag gag ctg acc agc ggc aag acc gag gtg ttc aac tat gag gaa       96
Lys Glu Glu Leu Thr Ser Gly Lys Thr Glu Val Phe Asn Tyr Glu Glu
            20                  25                  30

ctc ctc gag ttc gca gag ggc gac atc gcc aag gtc ttc gga ccc gag      144
Leu Leu Glu Phe Ala Glu Gly Asp Ile Ala Lys Val Phe Gly Pro Glu
        35                  40                  45

ttc gcc gtc atc gac aag tac ccg cgc cgc gtg cgc ctg ccc gcc cgc      192
Phe Ala Val Ile Asp Lys Tyr Pro Arg Arg Val Arg Leu Pro Ala Arg
    50                  55                  60

gag tac ctg ctc gtg acc cgc gtc acc ctc atg gac gcc gag gtc aac      240
Glu Tyr Leu Leu Val Thr Arg Val Thr Leu Met Asp Ala Glu Val Asn
65                  70                  75                  80

aac tac cgc gtc ggc gcc cgc atg gtc acc gag tac gat ctc ccc gtc      288
Asn Tyr Arg Val Gly Ala Arg Met Val Thr Glu Tyr Asp Leu Pro Val
                85                  90                  95

aac gga gag ctc tcc gag ggc gga gac tgc ccc tgg gcc gtc ctg gtc      336
Asn Gly Glu Leu Ser Glu Gly Gly Asp Cys Pro Trp Ala Val Leu Val
            100                 105                 110

gag agt ggc cag tgc gat ctc atg ctc atc tcc tac atg ggc att gac      384
Glu Ser Gly Gln Cys Asp Leu Met Leu Ile Ser Tyr Met Gly Ile Asp
        115                 120                 125

ttc cag aac cag ggc gac cgc gtc tac cgc ctg ctc aac acc acg ctc      432
Phe Gln Asn Gln Gly Asp Arg Val Tyr Arg Leu Leu Asn Thr Thr Leu
    130                 135                 140

acc ttt tac ggc gtg gcc cac gag ggc gag acc ctc gag tac gac att      480
Thr Phe Tyr Gly Val Ala His Glu Gly Glu Thr Leu Glu Tyr Asp Ile
145                 150                 155                 160

cgc gtc acc ggc ttc gcc aag cgt ctc gac ggc ggc atc tcc atg ttc      528
Arg Val Thr Gly Phe Ala Lys Arg Leu Asp Gly Gly Ile Ser Met Phe
                165                 170                 175

ttc ttc gag tac gac tgc tac gtc aac ggc cgc ctc ctc atc gag atg      576
Phe Phe Glu Tyr Asp Cys Tyr Val Asn Gly Arg Leu Leu Ile Glu Met
            180                 185                 190

cgc gat ggc tgc gcc ggc ttc ttc acc aac gag gag ctc gac gcc ggc      624
Arg Asp Gly Cys Ala Gly Phe Phe Thr Asn Glu Glu Leu Asp Ala Gly
        195                 200                 205

aag ggc gtc gtc ttc acc cgc ggc gac ctc gcc gcc cgc gcc aag atc      672
Lys Gly Val Val Phe Thr Arg Gly Asp Leu Ala Ala Arg Ala Lys Ile
    210                 215                 220

cca aag cag gac gtc tcc ccc tac gcc gtc gcc ccc tgc ctc cac aag      720
Pro Lys Gln Asp Val Ser Pro Tyr Ala Val Ala Pro Cys Leu His Lys
225                 230                 235                 240

acc aag ctc aac gaa aag gag atg cag acc ctc gtc gac aag gac tgg      768
Thr Lys Leu Asn Glu Lys Glu Met Gln Thr Leu Val Asp Lys Asp Trp
                245                 250                 255

gca tcc gtc ttt ggc tcc aag aac ggc atg ccg gaa atc aac tac aaa      816
Ala Ser Val Phe Gly Ser Lys Asn Gly Met Pro Glu Ile Asn Tyr Lys
            260                 265                 270

ctc tgc gcg cgt aag atg ctc atg att gac cgc gtc acc agc att gac      864
Leu Cys Ala Arg Lys Met Leu Met Ile Asp Arg Val Thr Ser Ile Asp
        275                 280                 285

cac aag ggc ggt gtc tac ggc ctc ggt cag ctc gtc ggt gaa aag atc      912
His Lys Gly Gly Val Tyr Gly Leu Gly Gln Leu Val Gly Glu Lys Ile
    290                 295                 300

ctc gag cgc gac cac tgg tac ttt ccc tgc cac ttt gtc aag gat cag      960
Leu Glu Arg Asp His Trp Tyr Phe Pro Cys His Phe Val Lys Asp Gln
305                 310                 315                 320
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gtc atg gcc gga tcc ctc gtc tcc gac ggc tgc agc cag atg ctc aag      1008
Val Met Ala Gly Ser Leu Val Ser Asp Gly Cys Ser Gln Met Leu Lys
                325                 330                 335

atg tac atg atc tgg ctc ggc ctc cac ctc acc acc gga ccc ttt gac      1056
Met Tyr Met Ile Trp Leu Gly Leu His Leu Thr Thr Gly Pro Phe Asp
            340                 345                 350

ttc cgc ccg gtc aac ggc cac ccc aac aag gtc cgc tgc cgc ggc caa      1104
Phe Arg Pro Val Asn Gly His Pro Asn Lys Val Arg Cys Arg Gly Gln
        355                 360                 365

atc tcc ccg cac aag ggc aag ctc gtc tac gtc atg gag atc aag gag      1152
Ile Ser Pro His Lys Gly Lys Leu Val Tyr Val Met Glu Ile Lys Glu
    370                 375                 380

atg ggc ttc gac gag gac aac gac ccg tac gcc att gcc gac gtc aac      1200
Met Gly Phe Asp Glu Asp Asn Asp Pro Tyr Ala Ile Ala Asp Val Asn
385                 390                 395                 400

atc att gat gtc gac ttc gaa aag ggc cag gac ttt agc ctc gac cgc      1248
Ile Ile Asp Val Asp Phe Glu Lys Gly Gln Asp Phe Ser Leu Asp Arg
                405                 410                 415

atc agc gac tac ggc aag ggc gac ctc aac aag aag atc gtc gtc gac      1296
Ile Ser Asp Tyr Gly Lys Gly Asp Leu Asn Lys Lys Ile Val Val Asp
            420                 425                 430

ttt aag ggc atc gct ctc aag atg cag aag cgc tcc acc aac aag aac      1344
Phe Lys Gly Ile Ala Leu Lys Met Gln Lys Arg Ser Thr Asn Lys Asn
        435                 440                 445

ccc tcc aag gtt cag ccc gtc ttt gcc aac ggc gcc gcc act gtc ggc      1392
Pro Ser Lys Val Gln Pro Val Phe Ala Asn Gly Ala Ala Thr Val Gly
    450                 455                 460

ccc gag gcc tcc aag gct tcc tcc ggc gcc agc gcc agc gcc agc gcc      1440
Pro Glu Ala Ser Lys Ala Ser Ser Gly Ala Ser Ala Ser Ala Ser Ala
465                 470                 475                 480

gcc ccg gcc aag cct gcc ttc agc gcc gat gtt ctt gcg ccc aag ccc      1488
Ala Pro Ala Lys Pro Ala Phe Ser Ala Asp Val Leu Ala Pro Lys Pro
                485                 490                 495

gtt gcc ctt ccc gag cac atc ctc aag ggc gac gcc ctc gcc ccc aag      1536
Val Ala Leu Pro Glu His Ile Leu Lys Gly Asp Ala Leu Ala Pro Lys
            500                 505                 510

gag atg tcc tgg cac ccc atg gcc cgc atc ccg ggc aac ccg acg ccc      1584
Glu Met Ser Trp His Pro Met Ala Arg Ile Pro Gly Asn Pro Thr Pro
        515                 520                 525

tct ttt gcg ccc tcg gcc tac aag ccg cgc aac atc gcc ttt acg ccc      1632
Ser Phe Ala Pro Ser Ala Tyr Lys Pro Arg Asn Ile Ala Phe Thr Pro
    530                 535                 540

ttc ccc ggc aac ccc aac gat aac gac cac acc ccg ggc aag atg ccg      1680
Phe Pro Gly Asn Pro Asn Asp Asn Asp His Thr Pro Gly Lys Met Pro
545                 550                 555                 560

ctc acc tgg ttc aac atg gcc gag ttc atg gcc ggc aag gtc agc atg      1728
Leu Thr Trp Phe Asn Met Ala Glu Phe Met Ala Gly Lys Val Ser Met
                565                 570                 575

tgc ctc ggc ccc gag ttc gcc aag ttc gac gac tcg aac acc agc cgc      1776
Cys Leu Gly Pro Glu Phe Ala Lys Phe Asp Asp Ser Asn Thr Ser Arg
            580                 585                 590

agc ccc gct tgg gac ctc gct ctc gtc acc cgc gcc gtg tct gtg tct      1824
Ser Pro Ala Trp Asp Leu Ala Leu Val Thr Arg Ala Val Ser Val Ser
        595                 600                 605

gac ctc aag cac gtc aac tac cgc aac atc gac ctc gac ccc tcc aag      1872
Asp Leu Lys His Val Asn Tyr Arg Asn Ile Asp Leu Asp Pro Ser Lys
    610                 615                 620

ggt acc atg gtc ggc gag ttc gac tgc ccc gcg gac gcc tgg ttc tac      1920
Gly Thr Met Val Gly Glu Phe Asp Cys Pro Ala Asp Ala Trp Phe Tyr
625                 630                 635                 640
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aag ggc gcc tgc aac gat gcc cac atg ccg tac tcg atc ctc atg gag     1968
Lys Gly Ala Cys Asn Asp Ala His Met Pro Tyr Ser Ile Leu Met Glu
                645                 650                 655

atc gcc ctc cag acc tcg ggt gtg ctc acc tcg gtg ctc aag gcg ccc     2016
Ile Ala Leu Gln Thr Ser Gly Val Leu Thr Ser Val Leu Lys Ala Pro
            660                 665                 670

ctg acc atg gag aag gac gac atc ctc ttc cgc aac ctc gac gcc aac     2064
Leu Thr Met Glu Lys Asp Asp Ile Leu Phe Arg Asn Leu Asp Ala Asn
        675                 680                 685

gcc gag ttc gtg cgc gcc gac ctc gac tac cgc ggc aag act atc cgc     2112
Ala Glu Phe Val Arg Ala Asp Leu Asp Tyr Arg Gly Lys Thr Ile Arg
    690                 695                 700

aac gtc acc aag tgc act ggc tac agc atg ctc ggc gag atg ggc gtc     2160
Asn Val Thr Lys Cys Thr Gly Tyr Ser Met Leu Gly Glu Met Gly Val
705                 710                 715                 720

cac cgc ttc acc ttt gag ctc tac gtc gat gat gtg ctc ttt tac aag     2208
His Arg Phe Thr Phe Glu Leu Tyr Val Asp Asp Val Leu Phe Tyr Lys
                725                 730                 735

ggc tcg acc tcg ttc ggc tgg ttc gtg ccc gag gtc ttt gcc gcc cag     2256
Gly Ser Thr Ser Phe Gly Trp Phe Val Pro Glu Val Phe Ala Ala Gln
            740                 745                 750

gcc ggc ctc gac aac ggc cgc aag tcg gag ccc tgg ttc att gag aac     2304
Ala Gly Leu Asp Asn Gly Arg Lys Ser Glu Pro Trp Phe Ile Glu Asn
        755                 760                 765

aag gtt ccg gcc tcg cag gtc tcc tcc ttt gac gtg cgc ccc aac ggc     2352
Lys Val Pro Ala Ser Gln Val Ser Ser Phe Asp Val Arg Pro Asn Gly
    770                 775                 780

agc ggc cgc acc gcc atc ttc gcc aac gcc ccc agc ggc gcc cag ctc     2400
Ser Gly Arg Thr Ala Ile Phe Ala Asn Ala Pro Ser Gly Ala Gln Leu
785                 790                 795                 800

aac cgc cgc acg gac cag ggc cag tac ctc gac gcc gtc gac att gtc     2448
Asn Arg Arg Thr Asp Gln Gly Gln Tyr Leu Asp Ala Val Asp Ile Val
                805                 810                 815

tcc ggc agc ggc aag aag agc ctc ggc tac gcc cac ggt tcc aag acg     2496
Ser Gly Ser Gly Lys Lys Ser Leu Gly Tyr Ala His Gly Ser Lys Thr
            820                 825                 830

gtc aac ccg aac gac tgg ttc ttc tcg tgc cac ttt tgg ttt gac tcg     2544
Val Asn Pro Asn Asp Trp Phe Phe Ser Cys His Phe Trp Phe Asp Ser
        835                 840                 845

gtc atg ccc gga agt ctc ggt gtc gag tcc atg ttc cag ctc gtc gag     2592
Val Met Pro Gly Ser Leu Gly Val Glu Ser Met Phe Gln Leu Val Glu
    850                 855                 860

gcc atc gcc gcc cac gag gat ctc gct ggc aaa gca cgg cat tgc caa     2640
Ala Ile Ala Ala His Glu Asp Leu Ala Gly Lys Ala Arg His Cys Gln
865                 870                 875                 880

ccc cac ctt tgt gca cgc ccc cgg gca aga tca agc tgg aag tac cgc     2688
Pro His Leu Cys Ala Arg Pro Arg Ala Arg Ser Ser Trp Lys Tyr Arg
                885                 890                 895

ggc cag ctc acg ccc aag agc aag aag atg gac tcg gag gtc cac atc     2736
Gly Gln Leu Thr Pro Lys Ser Lys Lys Met Asp Ser Glu Val His Ile
            900                 905                 910

gtg tcc gtg gac gcc cac gac ggc gtt gtc gac ctc gtc gcc gac ggc     2784
Val Ser Val Asp Ala His Asp Gly Val Val Asp Leu Val Ala Asp Gly
        915                 920                 925

ttc ctc tgg gcc gac agc ctc cgc gtc tac tcg gtg agc aac att cgc     2832
Phe Leu Trp Ala Asp Ser Leu Arg Val Tyr Ser Val Ser Asn Ile Arg
    930                 935                 940

gtg cgc atc gcc tcc ggt gag gcc cct gcc gcc gcc tcc tcc gcc gcc     2880
Val Arg Ile Ala Ser Gly Glu Ala Pro Ala Ala Ala Ser Ser Ala Ala
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945                 950                 955                 960

tct gtg ggc tcc tcg gct tcg tcc gtc gag cgc acg cgc tcg agc ccc      2928
Ser Val Gly Ser Ser Ala Ser Ser Val Glu Arg Thr Arg Ser Ser Pro
                965                 970                 975

gct gtc gcc tcc ggc ccg gcc cag acc atc gac ctc aag cag ctc aag      2976
Ala Val Ala Ser Gly Pro Ala Gln Thr Ile Asp Leu Lys Gln Leu Lys
            980                 985                 990

acc gag ctc ctc gag ctc gat gcc  ccg ctc tac ctc tcg  cag gac ccg    3024
Thr Glu Leu Leu Glu Leu Asp Ala  Pro Leu Tyr Leu Ser  Gln Asp Pro
        995                 1000                1005

acc agc  ggc cag ctc aag aag  cac acc gac gtg gcc  tcc ggc cag       3069
Thr Ser  Gly Gln Leu Lys Lys  His Thr Asp Val Ala  Ser Gly Gln
    1010                1015                1020

gcc acc  atc gtg cag ccc tgc  acg ctc ggc gac ctc  ggt gac cgc       3114
Ala Thr  Ile Val Gln Pro Cys  Thr Leu Gly Asp Leu  Gly Asp Arg
    1025                1030                1035

tcc ttc  atg gag acc tac ggc  gtc gtc gcc ccg ctg  tac acg ggc       3159
Ser Phe  Met Glu Thr Tyr Gly  Val Val Ala Pro Leu  Tyr Thr Gly
    1040                1045                1050

gcc atg  gcc aag ggc att gcc  tcg gcg gac ctc gtc  atc gcc gcc       3204
Ala Met  Ala Lys Gly Ile Ala  Ser Ala Asp Leu Val  Ile Ala Ala
    1055                1060                1065

ggc aag  cgc aag atc ctc ggc  tcc ttt ggc gcc ggc  ggc ctc ccc       3249
Gly Lys  Arg Lys Ile Leu Gly  Ser Phe Gly Ala Gly  Gly Leu Pro
    1070                1075                1080

atg cac  cac gtg cgc gcc gcc  ctc gag aag atc cag  gcc gcc ctg       3294
Met His  His Val Arg Ala Ala  Leu Glu Lys Ile Gln  Ala Ala Leu
    1085                1090                1095

cct cag  ggc ccc tac gcc gtc  aac ctc atc cac tcg  cct ttt gac       3339
Pro Gln  Gly Pro Tyr Ala Val  Asn Leu Ile His Ser  Pro Phe Asp
    1100                1105                1110

agc aac  ctc gag aag ggc aac  gtc gat ctc ttc ctc  gag aag ggc       3384
Ser Asn  Leu Glu Lys Gly Asn  Val Asp Leu Phe Leu  Glu Lys Gly
    1115                1120                1125

gtc act  gtg gtg gag gcc tcg  gca ttc atg acc ctc  acc ccg cag       3429
Val Thr  Val Val Glu Ala Ser  Ala Phe Met Thr Leu  Thr Pro Gln
    1130                1135                1140

gtc gtg  cgc tac cgc gcc gcc  ggc ctc tcg cgc aac  gcc gac ggt       3474
Val Val  Arg Tyr Arg Ala Ala  Gly Leu Ser Arg Asn  Ala Asp Gly
    1145                1150                1155

tcg gtc  aac atc cgc aac cgc  atc atc ggc aag gtc  tcg cgc acc       3519
Ser Val  Asn Ile Arg Asn Arg  Ile Ile Gly Lys Val  Ser Arg Thr
    1160                1165                1170

gag ctc  gcc gag atg ttc atc  cgc ccg gcc ccg gag  cac ctc ctc       3564
Glu Leu  Ala Glu Met Phe Ile  Arg Pro Ala Pro Glu  His Leu Leu
    1175                1180                1185

gag aag  ctc atc gcc tcg ggc  gag atc acc cag gag  cag gcc gag       3609
Glu Lys  Leu Ile Ala Ser Gly  Glu Ile Thr Gln Glu  Gln Ala Glu
    1190                1195                1200

ctc gcg  cgc cgc gtt ccc gtc  gcc gac gat atc gct  gtc gag gct       3654
Leu Ala  Arg Arg Val Pro Val  Ala Asp Asp Ile Ala  Val Glu Ala
    1205                1210                1215

gac tcg  ggc ggc cac acc gac  aac cgc ccc atc cac  gtc atc ctc       3699
Asp Ser  Gly Gly His Thr Asp  Asn Arg Pro Ile His  Val Ile Leu
    1220                1225                1230

ccg ctc  atc atc aac ctc cgc  aac cgc ctg cac cgc  gag tgc ggc       3744
Pro Leu  Ile Ile Asn Leu Arg  Asn Arg Leu His Arg  Glu Cys Gly
    1235                1240                1245

tac ccc  gcg cac ctc cgc gtc  cgc gtt ggc gcc ggc  ggt ggc gtc       3789
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Tyr Pro  Ala His Leu Arg Val  Arg Val Gly Ala Gly  Gly Gly Val
    1250                 1255                 1260

ggc tgc  ccg cag gcc gcc gcc  gcc gcg ctc acc atg  ggc gcc gcc      3834
Gly Cys  Pro Gln Ala Ala Ala  Ala Ala Leu Thr Met  Gly Ala Ala
    1265                 1270                 1275

ttc atc  gtc acc ggc act gtc  aac cag gtc gcc aag  cag tcc ggc      3879
Phe Ile  Val Thr Gly Thr Val  Asn Gln Val Ala Lys  Gln Ser Gly
    1280                 1285                 1290

acc tgc  gac aac gtg cgc aag  cag ctc tcg cag gcc  acc tac tcg      3924
Thr Cys  Asp Asn Val Arg Lys  Gln Leu Ser Gln Ala  Thr Tyr Ser
    1295                 1300                 1305

gat atc  tgc atg gcc ccg gcc  gcc gac atg ttc gag  gag ggc gtc      3969
Asp Ile  Cys Met Ala Pro Ala  Ala Asp Met Phe Glu  Glu Gly Val
    1310                 1315                 1320

aag ctc  cag gtc ctc aag aag  gga acc atg ttc ccc  tcg cgc gcc      4014
Lys Leu  Gln Val Leu Lys Lys  Gly Thr Met Phe Pro  Ser Arg Ala
    1325                 1330                 1335

aac aag  ctc tac gag ctc ttt  tgc aag tac gac tcc  ttc gac tcc      4059
Asn Lys  Leu Tyr Glu Leu Phe  Cys Lys Tyr Asp Ser  Phe Asp Ser
    1340                 1345                 1350

atg cct  cct gcc gag ctc gag  cgc atc gag aag cgt  atc ttc aag      4104
Met Pro  Pro Ala Glu Leu Glu  Arg Ile Glu Lys Arg  Ile Phe Lys
    1355                 1360                 1365

cgc gca  ctc cag gag gtc tgg  gag gag acc aag gac  ttt tac att      4149
Arg Ala  Leu Gln Glu Val Trp  Glu Glu Thr Lys Asp  Phe Tyr Ile
    1370                 1375                 1380

aac ggt  ctc aag aac ccg gag  aag atc cag cgc gcc  gag cac gac      4194
Asn Gly  Leu Lys Asn Pro Glu  Lys Ile Gln Arg Ala  Glu His Asp
    1385                 1390                 1395

ccc aag  ctc aag atg tcg ctc  tgc ttc cgc tgg tac  ctt ggt ctt      4239
Pro Lys  Leu Lys Met Ser Leu  Cys Phe Arg Trp Tyr  Leu Gly Leu
    1400                 1405                 1410

gcc agc  cgc tgg gcc aac atg  ggc gcc ccg gac cgc  gtc atg gac      4284
Ala Ser  Arg Trp Ala Asn Met  Gly Ala Pro Asp Arg  Val Met Asp
    1415                 1420                 1425

tac cag  gtc tgg tgt ggc ccg  gcc att ggc gcc ttc  aac gac ttc      4329
Tyr Gln  Val Trp Cys Gly Pro  Ala Ile Gly Ala Phe  Asn Asp Phe
    1430                 1435                 1440

atc aag  ggc acc tac ctc gac  ccc gct gtc tcc aac  gag tac ccc      4374
Ile Lys  Gly Thr Tyr Leu Asp  Pro Ala Val Ser Asn  Glu Tyr Pro
    1445                 1450                 1455

tgt gtc  gtc cag atc aac ctg  caa atc ctc cgt ggt  gcc tgc tac      4419
Cys Val  Val Gln Ile Asn Leu  Gln Ile Leu Arg Gly  Ala Cys Tyr
    1460                 1465                 1470

ctg cgc  cgt ctc aac gcc ctg  cgc aac gac ccg cgc  att gac ctc      4464
Leu Arg  Arg Leu Asn Ala Leu  Arg Asn Asp Pro Arg  Ile Asp Leu
    1475                 1480                 1485

gag acc  gag gat gct gcc ttt  gtc tac gag ccc acc  aac gcg ctc      4509
Glu Thr  Glu Asp Ala Ala Phe  Val Tyr Glu Pro Thr  Asn Ala Leu
    1490                 1495                 1500


<210> SEQ ID NO 6
<211> LENGTH: 1503
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 6

Met Ala Leu Arg Val Lys Thr Asn Lys Lys Pro Cys Trp Glu Met Thr
1               5                   10                  15

Lys Glu Glu Leu Thr Ser Gly Lys Thr Glu Val Phe Asn Tyr Glu Glu
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            20                  25                  30

Leu Leu Glu Phe Ala Glu Gly Asp Ile Ala Lys Val Phe Gly Pro Glu
        35                  40                  45

Phe Ala Val Ile Asp Lys Tyr Pro Arg Arg Val Arg Leu Pro Ala Arg
    50                  55                  60

Glu Tyr Leu Leu Val Thr Arg Val Thr Leu Met Asp Ala Glu Val Asn
65                  70                  75                  80

Asn Tyr Arg Val Gly Ala Arg Met Val Thr Glu Tyr Asp Leu Pro Val
                85                  90                  95

Asn Gly Glu Leu Ser Glu Gly Gly Asp Cys Pro Trp Ala Val Leu Val
            100                 105                 110

Glu Ser Gly Gln Cys Asp Leu Met Leu Ile Ser Tyr Met Gly Ile Asp
        115                 120                 125

Phe Gln Asn Gln Gly Asp Arg Val Tyr Arg Leu Leu Asn Thr Thr Leu
    130                 135                 140

Thr Phe Tyr Gly Val Ala His Glu Gly Glu Thr Leu Glu Tyr Asp Ile
145                 150                 155                 160

Arg Val Thr Gly Phe Ala Lys Arg Leu Asp Gly Gly Ile Ser Met Phe
                165                 170                 175

Phe Phe Glu Tyr Asp Cys Tyr Val Asn Gly Arg Leu Leu Ile Glu Met
            180                 185                 190

Arg Asp Gly Cys Ala Gly Phe Phe Thr Asn Glu Glu Leu Asp Ala Gly
        195                 200                 205

Lys Gly Val Val Phe Thr Arg Gly Asp Leu Ala Ala Arg Ala Lys Ile
    210                 215                 220

Pro Lys Gln Asp Val Ser Pro Tyr Ala Val Ala Pro Cys Leu His Lys
225                 230                 235                 240

Thr Lys Leu Asn Glu Lys Glu Met Gln Thr Leu Val Asp Lys Asp Trp
                245                 250                 255

Ala Ser Val Phe Gly Ser Lys Asn Gly Met Pro Glu Ile Asn Tyr Lys
            260                 265                 270

Leu Cys Ala Arg Lys Met Leu Met Ile Asp Arg Val Thr Ser Ile Asp
        275                 280                 285

His Lys Gly Gly Val Tyr Gly Leu Gly Gln Leu Val Gly Glu Lys Ile
    290                 295                 300

Leu Glu Arg Asp His Trp Tyr Phe Pro Cys His Phe Val Lys Asp Gln
305                 310                 315                 320

Val Met Ala Gly Ser Leu Val Ser Asp Gly Cys Ser Gln Met Leu Lys
                325                 330                 335

Met Tyr Met Ile Trp Leu Gly Leu His Leu Thr Thr Gly Pro Phe Asp
            340                 345                 350

Phe Arg Pro Val Asn Gly His Pro Asn Lys Val Arg Cys Arg Gly Gln
        355                 360                 365

Ile Ser Pro His Lys Gly Lys Leu Val Tyr Val Met Glu Ile Lys Glu
    370                 375                 380

Met Gly Phe Asp Glu Asp Asn Asp Pro Tyr Ala Ile Ala Asp Val Asn
385                 390                 395                 400

Ile Ile Asp Val Asp Phe Glu Lys Gly Gln Asp Phe Ser Leu Asp Arg
                405                 410                 415

Ile Ser Asp Tyr Gly Lys Gly Asp Leu Asn Lys Lys Ile Val Val Asp
            420                 425                 430

Phe Lys Gly Ile Ala Leu Lys Met Gln Lys Arg Ser Thr Asn Lys Asn
        435                 440                 445
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Pro Ser Lys Val Gln Pro Val Phe Ala Asn Gly Ala Ala Thr Val Gly
    450                 455                 460

Pro Glu Ala Ser Lys Ala Ser Ser Gly Ala Ser Ala Ser Ala Ser Ala
465                 470                 475                 480

Ala Pro Ala Lys Pro Ala Phe Ser Ala Asp Val Leu Ala Pro Lys Pro
                485                 490                 495

Val Ala Leu Pro Glu His Ile Leu Lys Gly Asp Ala Leu Ala Pro Lys
            500                 505                 510

Glu Met Ser Trp His Pro Met Ala Arg Ile Pro Gly Asn Pro Thr Pro
        515                 520                 525

Ser Phe Ala Pro Ser Ala Tyr Lys Pro Arg Asn Ile Ala Phe Thr Pro
    530                 535                 540

Phe Pro Gly Asn Pro Asn Asp Asn Asp His Thr Pro Gly Lys Met Pro
545                 550                 555                 560

Leu Thr Trp Phe Asn Met Ala Glu Phe Met Ala Gly Lys Val Ser Met
                565                 570                 575

Cys Leu Gly Pro Glu Phe Ala Lys Phe Asp Asp Ser Asn Thr Ser Arg
            580                 585                 590

Ser Pro Ala Trp Asp Leu Ala Leu Val Thr Arg Ala Val Ser Val Ser
        595                 600                 605

Asp Leu Lys His Val Asn Tyr Arg Asn Ile Asp Leu Asp Pro Ser Lys
    610                 615                 620

Gly Thr Met Val Gly Glu Phe Asp Cys Pro Ala Asp Ala Trp Phe Tyr
625                 630                 635                 640

Lys Gly Ala Cys Asn Asp Ala His Met Pro Tyr Ser Ile Leu Met Glu
                645                 650                 655

Ile Ala Leu Gln Thr Ser Gly Val Leu Thr Ser Val Leu Lys Ala Pro
            660                 665                 670

Leu Thr Met Glu Lys Asp Asp Ile Leu Phe Arg Asn Leu Asp Ala Asn
        675                 680                 685

Ala Glu Phe Val Arg Ala Asp Leu Asp Tyr Arg Gly Lys Thr Ile Arg
    690                 695                 700

Asn Val Thr Lys Cys Thr Gly Tyr Ser Met Leu Gly Glu Met Gly Val
705                 710                 715                 720

His Arg Phe Thr Phe Glu Leu Tyr Val Asp Asp Val Leu Phe Tyr Lys
                725                 730                 735

Gly Ser Thr Ser Phe Gly Trp Phe Val Pro Glu Val Phe Ala Ala Gln
            740                 745                 750

Ala Gly Leu Asp Asn Gly Arg Lys Ser Glu Pro Trp Phe Ile Glu Asn
        755                 760                 765

Lys Val Pro Ala Ser Gln Val Ser Ser Phe Asp Val Arg Pro Asn Gly
    770                 775                 780

Ser Gly Arg Thr Ala Ile Phe Ala Asn Ala Pro Ser Gly Ala Gln Leu
785                 790                 795                 800

Asn Arg Arg Thr Asp Gln Gly Gln Tyr Leu Asp Ala Val Asp Ile Val
                805                 810                 815

Ser Gly Ser Gly Lys Lys Ser Leu Gly Tyr Ala His Gly Ser Lys Thr
            820                 825                 830

Val Asn Pro Asn Asp Trp Phe Phe Ser Cys His Phe Trp Phe Asp Ser
        835                 840                 845

Val Met Pro Gly Ser Leu Gly Val Glu Ser Met Phe Gln Leu Val Glu
    850                 855                 860
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Ala Ile Ala Ala His Glu Asp Leu Ala Gly Lys Ala Arg His Cys Gln
865                 870                 875                 880

Pro His Leu Cys Ala Arg Pro Arg Ala Arg Ser Ser Trp Lys Tyr Arg
                885                 890                 895

Gly Gln Leu Thr Pro Lys Ser Lys Lys Met Asp Ser Glu Val His Ile
            900                 905                 910

Val Ser Val Asp Ala His Asp Gly Val Val Asp Leu Val Ala Asp Gly
        915                 920                 925

Phe Leu Trp Ala Asp Ser Leu Arg Val Tyr Ser Val Ser Asn Ile Arg
    930                 935                 940

Val Arg Ile Ala Ser Gly Glu Ala Pro Ala Ala Ala Ser Ser Ala Ala
945                 950                 955                 960

Ser Val Gly Ser Ser Ala Ser Ser Val Glu Arg Thr Arg Ser Ser Pro
                965                 970                 975

Ala Val Ala Ser Gly Pro Ala Gln Thr Ile Asp Leu Lys Gln Leu Lys
            980                 985                 990

Thr Glu Leu Leu Glu Leu Asp Ala  Pro Leu Tyr Leu Ser  Gln Asp Pro
        995                 1000                1005

Thr Ser  Gly Gln Leu Lys Lys  His Thr Asp Val Ala  Ser Gly Gln
    1010                 1015                1020

Ala Thr  Ile Val Gln Pro Cys  Thr Leu Gly Asp Leu  Gly Asp Arg
    1025                 1030                1035

Ser Phe  Met Glu Thr Tyr Gly  Val Val Ala Pro Leu  Tyr Thr Gly
    1040                 1045                1050

Ala Met  Ala Lys Gly Ile Ala  Ser Ala Asp Leu Val  Ile Ala Ala
    1055                 1060                1065

Gly Lys  Arg Lys Ile Leu Gly  Ser Phe Gly Ala Gly  Gly Leu Pro
    1070                 1075                1080

Met His  His Val Arg Ala Ala  Leu Glu Lys Ile Gln  Ala Ala Leu
    1085                 1090                1095

Pro Gln  Gly Pro Tyr Ala Val  Asn Leu Ile His Ser  Pro Phe Asp
    1100                 1105                1110

Ser Asn  Leu Glu Lys Gly Asn  Val Asp Leu Phe Leu  Glu Lys Gly
    1115                 1120                1125

Val Thr  Val Val Glu Ala Ser  Ala Phe Met Thr Leu  Thr Pro Gln
    1130                 1135                1140

Val Val  Arg Tyr Arg Ala Ala  Gly Leu Ser Arg Asn  Ala Asp Gly
    1145                 1150                1155

Ser Val  Asn Ile Arg Asn Arg  Ile Ile Gly Lys Val  Ser Arg Thr
    1160                 1165                1170

Glu Leu  Ala Glu Met Phe Ile  Arg Pro Ala Pro Glu  His Leu Leu
    1175                 1180                1185

Glu Lys  Leu Ile Ala Ser Gly  Glu Ile Thr Gln Glu  Gln Ala Glu
    1190                 1195                1200

Leu Ala  Arg Arg Val Pro Val  Ala Asp Asp Ile Ala  Val Glu Ala
    1205                 1210                1215

Asp Ser  Gly Gly His Thr Asp  Asn Arg Pro Ile His  Val Ile Leu
    1220                 1225                1230

Pro Leu  Ile Ile Asn Leu Arg  Asn Arg Leu His Arg  Glu Cys Gly
    1235                 1240                1245

Tyr Pro  Ala His Leu Arg Val  Arg Val Gly Ala Gly  Gly Gly Val
    1250                 1255                1260

Gly Cys  Pro Gln Ala Ala Ala  Ala Ala Leu Thr Met  Gly Ala Ala
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    1265                1270                1275

Phe Ile  Val Thr Gly Thr Val  Asn Gln Val Ala Lys  Gln Ser Gly
    1280                1285                1290

Thr Cys  Asp Asn Val Arg Lys  Gln Leu Ser Gln Ala  Thr Tyr Ser
    1295                1300                1305

Asp Ile  Cys Met Ala Pro Ala  Ala Asp Met Phe Glu  Glu Gly Val
    1310                1315                1320

Lys Leu  Gln Val Leu Lys Lys  Gly Thr Met Phe Pro  Ser Arg Ala
    1325                1330                1335

Asn Lys  Leu Tyr Glu Leu Phe  Cys Lys Tyr Asp Ser  Phe Asp Ser
    1340                1345                1350

Met Pro  Pro Ala Glu Leu Glu  Arg Ile Glu Lys Arg  Ile Phe Lys
    1355                1360                1365

Arg Ala  Leu Gln Glu Val Trp  Glu Glu Thr Lys Asp  Phe Tyr Ile
    1370                1375                1380

Asn Gly  Leu Lys Asn Pro Glu  Lys Ile Gln Arg Ala  Glu His Asp
    1385                1390                1395

Pro Lys  Leu Lys Met Ser Leu  Cys Phe Arg Trp Tyr  Leu Gly Leu
    1400                1405                1410

Ala Ser  Arg Trp Ala Asn Met  Gly Ala Pro Asp Arg  Val Met Asp
    1415                1420                1425

Tyr Gln  Val Trp Cys Gly Pro  Ala Ile Gly Ala Phe  Asn Asp Phe
    1430                1435                1440

Ile Lys  Gly Thr Tyr Leu Asp  Pro Ala Val Ser Asn  Glu Tyr Pro
    1445                1450                1455

Cys Val  Val Gln Ile Asn Leu  Gln Ile Leu Arg Gly  Ala Cys Tyr
    1460                1465                1470

Leu Arg  Arg Leu Asn Ala Leu  Arg Asn Asp Pro Arg  Ile Asp Leu
    1475                1480                1485

Glu Thr  Glu Asp Ala Ala Phe  Val Tyr Glu Pro Thr  Asn Ala Leu
    1490                1495                1500


<210> SEQ ID NO 7
<211> LENGTH: 1500
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1500)

<400> SEQUENCE: 7

atg gcg gcc cgt ctg cag gag caa aag gga ggc gag atg gat acc cgc       48
Met Ala Ala Arg Leu Gln Glu Gln Lys Gly Gly Glu Met Asp Thr Arg
1               5                   10                  15

att gcc atc atc ggc atg tcg gcc atc ctc ccc tgc ggc acg acc gtg       96
Ile Ala Ile Ile Gly Met Ser Ala Ile Leu Pro Cys Gly Thr Thr Val
            20                  25                  30

cgc gag tcg tgg gag acc atc cgc gcc ggc atc gac tgc ctg tcg gat      144
Arg Glu Ser Trp Glu Thr Ile Arg Ala Gly Ile Asp Cys Leu Ser Asp
        35                  40                  45

ctc ccc gag gac cgc gtc gac gtg acg gcg tac ttt gac ccc gtc aag      192
Leu Pro Glu Asp Arg Val Asp Val Thr Ala Tyr Phe Asp Pro Val Lys
    50                  55                  60

acc acc aag gac aag atc tac tgc aag cgc ggt ggc ttc att ccc gag      240
Thr Thr Lys Asp Lys Ile Tyr Cys Lys Arg Gly Gly Phe Ile Pro Glu
65                  70                  75                  80

tac gac ttt gac gcc cgc gag ttc gga ctc aac atg ttc cag atg gag      288
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Tyr Asp Phe Asp Ala Arg Glu Phe Gly Leu Asn Met Phe Gln Met Glu
                85                  90                  95

gac tcg gac gca aac cag acc atc tcg ctt ctc aag gtc aag gag gcc      336
Asp Ser Asp Ala Asn Gln Thr Ile Ser Leu Leu Lys Val Lys Glu Ala
            100                 105                 110

ctc cag gac gcc ggc atc gac gcc ctc ggc aag gaa aag aag aac atc      384
Leu Gln Asp Ala Gly Ile Asp Ala Leu Gly Lys Glu Lys Lys Asn Ile
        115                 120                 125

ggc tgc gtg ctc ggc att ggc ggc ggc caa aag tcc agc cac gag ttc      432
Gly Cys Val Leu Gly Ile Gly Gly Gly Gln Lys Ser Ser His Glu Phe
    130                 135                 140

tac tcg cgc ctt aat tat gtt gtc gtg gag aag gtc ctc cgc aag atg      480
Tyr Ser Arg Leu Asn Tyr Val Val Val Glu Lys Val Leu Arg Lys Met
145                 150                 155                 160

ggc atg ccc gag gag gac gtc aag gtc gcc gtc gaa aag tac aag gcc      528
Gly Met Pro Glu Glu Asp Val Lys Val Ala Val Glu Lys Tyr Lys Ala
                165                 170                 175

aac ttc ccc gag tgg cgc ctc gac tcc ttc cct ggc ttc ctc ggc aac      576
Asn Phe Pro Glu Trp Arg Leu Asp Ser Phe Pro Gly Phe Leu Gly Asn
            180                 185                 190

gtc acc gcc ggt cgc tgc acc aac acc ttc aac ctc gac ggc atg aac      624
Val Thr Ala Gly Arg Cys Thr Asn Thr Phe Asn Leu Asp Gly Met Asn
        195                 200                 205

tgc gtt gtc gac gcc gca tgc gcc tcg tcc ctc atc gcc gtc aag gtc      672
Cys Val Val Asp Ala Ala Cys Ala Ser Ser Leu Ile Ala Val Lys Val
    210                 215                 220

gcc atc gac gag ctg ctc tac ggt gac tgc gac atg atg gtc acc ggt      720
Ala Ile Asp Glu Leu Leu Tyr Gly Asp Cys Asp Met Met Val Thr Gly
225                 230                 235                 240

gcc acc tgc acg gat aac tcc atc ggc atg tac atg gcc ttc tcc aag      768
Ala Thr Cys Thr Asp Asn Ser Ile Gly Met Tyr Met Ala Phe Ser Lys
                245                 250                 255

acc ccc gtg ttc tcc acg gac ccc agc gtg cgc gcc tac gac gaa aag      816
Thr Pro Val Phe Ser Thr Asp Pro Ser Val Arg Ala Tyr Asp Glu Lys
            260                 265                 270

aca aag ggc atg ctc atc ggc gag ggc tcc gcc atg ctc gtc ctc aag      864
Thr Lys Gly Met Leu Ile Gly Glu Gly Ser Ala Met Leu Val Leu Lys
        275                 280                 285

cgc tac gcc gac gcc gtc cgc gac ggc gat gag atc cac gct gtt att      912
Arg Tyr Ala Asp Ala Val Arg Asp Gly Asp Glu Ile His Ala Val Ile
    290                 295                 300

cgc ggc tgc gcc tcc tcc agt gat ggc aag gcc gcc ggc atc tac acg      960
Arg Gly Cys Ala Ser Ser Ser Asp Gly Lys Ala Ala Gly Ile Tyr Thr
305                 310                 315                 320

ccc acc att tcg ggc cag gag gag gcc ctc cgc cgc gcc tac aac cgc     1008
Pro Thr Ile Ser Gly Gln Glu Glu Ala Leu Arg Arg Ala Tyr Asn Arg
                325                 330                 335

gcc tgt gtc gac ccg gcc acc gtc act ctc gtc gag ggt cac ggc acc     1056
Ala Cys Val Asp Pro Ala Thr Val Thr Leu Val Glu Gly His Gly Thr
            340                 345                 350

ggt act ccc gtt ggc gac cgc atc gag ctc acc gcc ttg cgc aac ctc     1104
Gly Thr Pro Val Gly Asp Arg Ile Glu Leu Thr Ala Leu Arg Asn Leu
        355                 360                 365

ttt gac aag gcc tac ggc gag ggc aac acc gaa aag gtc gct gtg ggc     1152
Phe Asp Lys Ala Tyr Gly Glu Gly Asn Thr Glu Lys Val Ala Val Gly
    370                 375                 380

agc atc aag tcc agc atc ggc cat ctc aag gcc gtc gcc ggt ctc gcc     1200
Ser Ile Lys Ser Ser Ile Gly His Leu Lys Ala Val Ala Gly Leu Ala
385                 390                 395                 400
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ggt atg atc aag gtc atc atg gcg ctc aag cac aag act ctc ccg ggc      1248
Gly Met Ile Lys Val Ile Met Ala Leu Lys His Lys Thr Leu Pro Gly
            405                 410                 415

acc atc aac gtc gac aac cca ccc aac ctc tac gac aac acg ccc atc      1296
Thr Ile Asn Val Asp Asn Pro Pro Asn Leu Tyr Asp Asn Thr Pro Ile
            420                 425                 430

aac gag tcc tcg ctc tac att aac acc atg aac cgc ccc tgg ttc ccg      1344
Asn Glu Ser Ser Leu Tyr Ile Asn Thr Met Asn Arg Pro Trp Phe Pro
        435                 440                 445

ccc cct ggt gtg ccc cgc cgc gcc ggc att tcg agc ttt ggc ttt ggt      1392
Pro Pro Gly Val Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly
    450                 455                 460

ggc gcc aac tac cac gcc gtc ctc gag gag gcc gag ccc gag cac acg      1440
Gly Ala Asn Tyr His Ala Val Leu Glu Glu Ala Glu Pro Glu His Thr
465                 470                 475                 480

acc gcg tac cgc ctc aac aag cgc ccg cag ccc gtg ctc atg atg gcc      1488
Thr Ala Tyr Arg Leu Asn Lys Arg Pro Gln Pro Val Leu Met Met Ala
                485                 490                 495

gcc acg ccc gcg                                                      1500
Ala Thr Pro Ala
            500


<210> SEQ ID NO 8
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 8

Met Ala Ala Arg Leu Gln Glu Gln Lys Gly Gly Glu Met Asp Thr Arg
1               5                   10                  15

Ile Ala Ile Ile Gly Met Ser Ala Ile Leu Pro Cys Gly Thr Thr Val
            20                  25                  30

Arg Glu Ser Trp Glu Thr Ile Arg Ala Gly Ile Asp Cys Leu Ser Asp
        35                  40                  45

Leu Pro Glu Asp Arg Val Asp Val Thr Ala Tyr Phe Asp Pro Val Lys
    50                  55                  60

Thr Thr Lys Asp Lys Ile Tyr Cys Lys Arg Gly Gly Phe Ile Pro Glu
65                  70                  75                  80

Tyr Asp Phe Asp Ala Arg Glu Phe Gly Leu Asn Met Phe Gln Met Glu
                85                  90                  95

Asp Ser Asp Ala Asn Gln Thr Ile Ser Leu Leu Lys Val Lys Glu Ala
            100                 105                 110

Leu Gln Asp Ala Gly Ile Asp Ala Leu Gly Lys Glu Lys Lys Asn Ile
        115                 120                 125

Gly Cys Val Leu Gly Ile Gly Gly Gly Gln Lys Ser Ser His Glu Phe
    130                 135                 140

Tyr Ser Arg Leu Asn Tyr Val Val Val Glu Lys Val Leu Arg Lys Met
145                 150                 155                 160

Gly Met Pro Glu Glu Asp Val Lys Val Ala Val Glu Lys Tyr Lys Ala
                165                 170                 175

Asn Phe Pro Glu Trp Arg Leu Asp Ser Phe Pro Gly Phe Leu Gly Asn
            180                 185                 190

Val Thr Ala Gly Arg Cys Thr Asn Thr Phe Asn Leu Asp Gly Met Asn
        195                 200                 205

Cys Val Val Asp Ala Ala Cys Ala Ser Ser Leu Ile Ala Val Lys Val
    210                 215                 220

Ala Ile Asp Glu Leu Leu Tyr Gly Asp Cys Asp Met Met Val Thr Gly
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225                 230                 235                 240

Ala Thr Cys Thr Asp Asn Ser Ile Gly Met Tyr Met Ala Phe Ser Lys
                245                 250                 255

Thr Pro Val Phe Ser Thr Asp Pro Ser Val Arg Ala Tyr Asp Glu Lys
            260                 265                 270

Thr Lys Gly Met Leu Ile Gly Glu Gly Ser Ala Met Leu Val Leu Lys
        275                 280                 285

Arg Tyr Ala Asp Ala Val Arg Asp Gly Asp Glu Ile His Ala Val Ile
    290                 295                 300

Arg Gly Cys Ala Ser Ser Ser Asp Gly Lys Ala Ala Gly Ile Tyr Thr
305                 310                 315                 320

Pro Thr Ile Ser Gly Gln Glu Glu Ala Leu Arg Arg Ala Tyr Asn Arg
                325                 330                 335

Ala Cys Val Asp Pro Ala Thr Val Thr Leu Val Glu Gly His Gly Thr
            340                 345                 350

Gly Thr Pro Val Gly Asp Arg Ile Glu Leu Thr Ala Leu Arg Asn Leu
        355                 360                 365

Phe Asp Lys Ala Tyr Gly Glu Gly Asn Thr Glu Lys Val Ala Val Gly
    370                 375                 380

Ser Ile Lys Ser Ser Ile Gly His Leu Lys Ala Val Ala Gly Leu Ala
385                 390                 395                 400

Gly Met Ile Lys Val Ile Met Ala Leu Lys His Lys Thr Leu Pro Gly
                405                 410                 415

Thr Ile Asn Val Asp Asn Pro Pro Asn Leu Tyr Asp Asn Thr Pro Ile
            420                 425                 430

Asn Glu Ser Ser Leu Tyr Ile Asn Thr Met Asn Arg Pro Trp Phe Pro
        435                 440                 445

Pro Pro Gly Val Pro Arg Arg Ala Gly Ile Ser Ser Phe Gly Phe Gly
    450                 455                 460

Gly Ala Asn Tyr His Ala Val Leu Glu Glu Ala Glu Pro Glu His Thr
465                 470                 475                 480

Thr Ala Tyr Arg Leu Asn Lys Arg Pro Gln Pro Val Leu Met Met Ala
                485                 490                 495

Ala Thr Pro Ala
            500


<210> SEQ ID NO 9
<211> LENGTH: 1278
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1278)

<400> SEQUENCE: 9

gat gtc acc aag gag gcc tgg cgc ctc ccc cgc gag ggc gtc agc ttc       48
Asp Val Thr Lys Glu Ala Trp Arg Leu Pro Arg Glu Gly Val Ser Phe
1               5                   10                  15

cgc gcc aag ggc atc gcc acc aac ggc gct gtc gcc gcg ctc ttc tcc       96
Arg Ala Lys Gly Ile Ala Thr Asn Gly Ala Val Ala Ala Leu Phe Ser
            20                  25                  30

ggc cag ggc gcg cag tac acg cac atg ttt agc gag gtg gcc atg aac      144
Gly Gln Gly Ala Gln Tyr Thr His Met Phe Ser Glu Val Ala Met Asn
        35                  40                  45

tgg ccc cag ttc cgc cag agc att gcc gcc atg gac gcc gcc cag tcc      192
Trp Pro Gln Phe Arg Gln Ser Ile Ala Ala Met Asp Ala Ala Gln Ser
    50                  55                  60
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aag gtc gct gga agc gac aag gac ttt gag cgc gtc tcc cag gtc ctc      240
Lys Val Ala Gly Ser Asp Lys Asp Phe Glu Arg Val Ser Gln Val Leu
65                  70                  75                  80

tac ccg cgc aag ccg tac gag cgt gag ccc gag cag aac ccc aag aag      288
Tyr Pro Arg Lys Pro Tyr Glu Arg Glu Pro Glu Gln Asn Pro Lys Lys
                85                  90                  95

atc tcc ctc acc gcc tac tcg cag ccc tcg acc ctg gcc tgc gct ctc      336
Ile Ser Leu Thr Ala Tyr Ser Gln Pro Ser Thr Leu Ala Cys Ala Leu
            100                 105                 110

ggt gcc ttt gag atc ttc aag gag gcc ggc ttc acc ccg gac ttt gcc      384
Gly Ala Phe Glu Ile Phe Lys Glu Ala Gly Phe Thr Pro Asp Phe Ala
        115                 120                 125

gcc ggc cat tcg ctc ggt gag ttc gcc gcc ctc tac gcc gcg ggc tgc      432
Ala Gly His Ser Leu Gly Glu Phe Ala Ala Leu Tyr Ala Ala Gly Cys
    130                 135                 140

gtc gac cgc gac gag ctc ttt gag ctt gtc tgc cgc cgc gcc cgc atc      480
Val Asp Arg Asp Glu Leu Phe Glu Leu Val Cys Arg Arg Ala Arg Ile
145                 150                 155                 160

atg ggc ggc aag gac gca ccg gcc acc ccc aag gga tgc atg gcc gcc      528
Met Gly Gly Lys Asp Ala Pro Ala Thr Pro Lys Gly Cys Met Ala Ala
                165                 170                 175

gtc att ggc ccc aac gcc gag aac atc aag gtc cag gcc gcc aac gtc      576
Val Ile Gly Pro Asn Ala Glu Asn Ile Lys Val Gln Ala Ala Asn Val
            180                 185                 190

tgg ctc ggc aac tcc aac tcg cct tcg cag acc gtc atc acc ggc tcc      624
Trp Leu Gly Asn Ser Asn Ser Pro Ser Gln Thr Val Ile Thr Gly Ser
        195                 200                 205

gtc gaa ggt atc cag gcc gag agc gcc cgc ctc cag aag gag ggc ttc      672
Val Glu Gly Ile Gln Ala Glu Ser Ala Arg Leu Gln Lys Glu Gly Phe
    210                 215                 220

cgc gtc gtg cct ctt gcc tgc gag agc gcc ttc cac tcg ccc cag atg      720
Arg Val Val Pro Leu Ala Cys Glu Ser Ala Phe His Ser Pro Gln Met
225                 230                 235                 240

gag aac gcc tcg tcg gcc ttc aag gac gtc atc tcc aag gtc tcc ttc      768
Glu Asn Ala Ser Ser Ala Phe Lys Asp Val Ile Ser Lys Val Ser Phe
                245                 250                 255

cgc acc ccc aag gcc gag acc aag ctc ttc agc aac gtc tct ggc gag      816
Arg Thr Pro Lys Ala Glu Thr Lys Leu Phe Ser Asn Val Ser Gly Glu
            260                 265                 270

acc tac ccc acg gac gcc cgc gag atg ctt acg cag cac atg acc agc      864
Thr Tyr Pro Thr Asp Ala Arg Glu Met Leu Thr Gln His Met Thr Ser
        275                 280                 285

agc gtc aag ttc ctc acc cag gtc cgc aac atg cac cag gcc ggt gcg      912
Ser Val Lys Phe Leu Thr Gln Val Arg Asn Met His Gln Ala Gly Ala
    290                 295                 300

cgc atc ttt gtc gag ttc gga ccc aag cag gtg ctc tcc aag ctt gtc      960
Arg Ile Phe Val Glu Phe Gly Pro Lys Gln Val Leu Ser Lys Leu Val
305                 310                 315                 320

tcc gag acc ctc aag gat gac ccc tcg gtt gtc acc gtc tct gtc aac     1008
Ser Glu Thr Leu Lys Asp Asp Pro Ser Val Val Thr Val Ser Val Asn
                325                 330                 335

ccg gcc tcg ggc acg gat tcg gac atc cag ctc cgc gac gcg gcc gtc     1056
Pro Ala Ser Gly Thr Asp Ser Asp Ile Gln Leu Arg Asp Ala Ala Val
            340                 345                 350

cag ctc gtt gtc gct ggc gtc aac ctt cag ggc ttt gac aag tgg gac     1104
Gln Leu Val Val Ala Gly Val Asn Leu Gln Gly Phe Asp Lys Trp Asp
        355                 360                 365

gcc ccc gat gcc acc cgc atg cag gcc atc aag aag aag cgc act acc     1152
Ala Pro Asp Ala Thr Arg Met Gln Ala Ile Lys Lys Lys Arg Thr Thr
```

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    370                 375                 380

ctc cgc ctt tcg gcc gcc acc tac gtc tcg gac aag acc aag aag gtc      1200
Leu Arg Leu Ser Ala Ala Thr Tyr Val Ser Asp Lys Thr Lys Lys Val
385                 390                 395                 400

cgc gac gcc gcc atg aac gat ggc cgc tgc gtc acc tac ctc aag ggc      1248
Arg Asp Ala Ala Met Asn Asp Gly Arg Cys Val Thr Tyr Leu Lys Gly
                405                 410                 415

gcc gca ccg ctc atc aag gcc ccg gag ccc                              1278
Ala Ala Pro Leu Ile Lys Ala Pro Glu Pro
            420                 425


<210> SEQ ID NO 10
<211> LENGTH: 426
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 10

Asp Val Thr Lys Glu Ala Trp Arg Leu Pro Arg Glu Gly Val Ser Phe
1               5                   10                  15

Arg Ala Lys Gly Ile Ala Thr Asn Gly Ala Val Ala Ala Leu Phe Ser
            20                  25                  30

Gly Gln Gly Ala Gln Tyr Thr His Met Phe Ser Glu Val Ala Met Asn
        35                  40                  45

Trp Pro Gln Phe Arg Gln Ser Ile Ala Ala Met Asp Ala Ala Gln Ser
    50                  55                  60

Lys Val Ala Gly Ser Asp Lys Asp Phe Glu Arg Val Ser Gln Val Leu
65                  70                  75                  80

Tyr Pro Arg Lys Pro Tyr Glu Arg Glu Pro Glu Gln Asn Pro Lys Lys
                85                  90                  95

Ile Ser Leu Thr Ala Tyr Ser Gln Pro Ser Thr Leu Ala Cys Ala Leu
            100                 105                 110

Gly Ala Phe Glu Ile Phe Lys Glu Ala Gly Phe Thr Pro Asp Phe Ala
        115                 120                 125

Ala Gly His Ser Leu Gly Glu Phe Ala Ala Leu Tyr Ala Ala Gly Cys
    130                 135                 140

Val Asp Arg Asp Glu Leu Phe Glu Leu Val Cys Arg Arg Ala Arg Ile
145                 150                 155                 160

Met Gly Gly Lys Asp Ala Pro Ala Thr Pro Lys Gly Cys Met Ala Ala
                165                 170                 175

Val Ile Gly Pro Asn Ala Glu Asn Ile Lys Val Gln Ala Ala Asn Val
            180                 185                 190

Trp Leu Gly Asn Ser Asn Ser Pro Ser Gln Thr Val Ile Thr Gly Ser
        195                 200                 205

Val Glu Gly Ile Gln Ala Glu Ser Ala Arg Leu Gln Lys Glu Gly Phe
    210                 215                 220

Arg Val Val Pro Leu Ala Cys Glu Ser Ala Phe His Ser Pro Gln Met
225                 230                 235                 240

Glu Asn Ala Ser Ser Ala Phe Lys Asp Val Ile Ser Lys Val Ser Phe
                245                 250                 255

Arg Thr Pro Lys Ala Glu Thr Lys Leu Phe Ser Asn Val Ser Gly Glu
            260                 265                 270

Thr Tyr Pro Thr Asp Ala Arg Glu Met Leu Thr Gln His Met Thr Ser
        275                 280                 285

Ser Val Lys Phe Leu Thr Gln Val Arg Asn Met His Gln Ala Gly Ala
    290                 295                 300
```

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Arg Ile Phe Val Glu Phe Gly Pro Lys Gln Val Leu Ser Lys Leu Val
305                 310                 315                 320

Ser Glu Thr Leu Lys Asp Asp Pro Ser Val Val Thr Val Ser Val Asn
                325                 330                 335

Pro Ala Ser Gly Thr Asp Ser Asp Ile Gln Leu Arg Asp Ala Ala Val
            340                 345                 350

Gln Leu Val Val Ala Gly Val Asn Leu Gln Gly Phe Asp Lys Trp Asp
        355                 360                 365

Ala Pro Asp Ala Thr Arg Met Gln Ala Ile Lys Lys Lys Arg Thr Thr
    370                 375                 380

Leu Arg Leu Ser Ala Ala Thr Tyr Val Ser Asp Lys Thr Lys Lys Val
385                 390                 395                 400

Arg Asp Ala Ala Met Asn Asp Gly Arg Cys Val Thr Tyr Leu Lys Gly
                405                 410                 415

Ala Ala Pro Leu Ile Lys Ala Pro Glu Pro
            420                 425


<210> SEQ ID NO 11
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: X = any amino acid

<400> SEQUENCE: 11

Gly His Ser Xaa Gly
1               5


<210> SEQ ID NO 12
<211> LENGTH: 258
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(258)

<400> SEQUENCE: 12

gct gtc tcg aac gag ctt ctt gag aag gcc gag act gtc gtc atg gag       48
Ala Val Ser Asn Glu Leu Leu Glu Lys Ala Glu Thr Val Val Met Glu
1               5                   10                  15

gtc ctc gcc gcc aag acc ggc tac gag acc gac atg atc gag gct gac       96
Val Leu Ala Ala Lys Thr Gly Tyr Glu Thr Asp Met Ile Glu Ala Asp
            20                  25                  30

atg gag ctc gag acc gag ctc ggc att gac tcc atc aag cgt gtc gag      144
Met Glu Leu Glu Thr Glu Leu Gly Ile Asp Ser Ile Lys Arg Val Glu
        35                  40                  45

atc ctc tcc gag gtc cag gcc atg ctc aat gtc gag gcc aag gat gtc      192
Ile Leu Ser Glu Val Gln Ala Met Leu Asn Val Glu Ala Lys Asp Val
    50                  55                  60

gat gcc ctc agc cgc act cgc act gtt ggt gag gtt gtc aac gcc atg      240
Asp Ala Leu Ser Arg Thr Arg Thr Val Gly Glu Val Val Asn Ala Met
65                  70                  75                  80

aag gcc gag atc gct ggc                                              258
Lys Ala Glu Ile Ala Gly
                85


<210> SEQ ID NO 13
<211> LENGTH: 86
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.
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<400> SEQUENCE: 13

```
Ala Val Ser Asn Glu Leu Leu Glu Lys Ala Glu Thr Val Val Met Glu
1               5                   10                  15

Val Leu Ala Ala Lys Thr Gly Tyr Glu Thr Asp Met Ile Glu Ala Asp
            20                  25                  30

Met Glu Leu Glu Thr Glu Leu Gly Ile Asp Ser Ile Lys Arg Val Glu
        35                  40                  45

Ile Leu Ser Glu Val Gln Ala Met Leu Asn Val Glu Ala Lys Asp Val
    50                  55                  60

Asp Ala Leu Ser Arg Thr Arg Thr Val Gly Glu Val Val Asn Ala Met
65                  70                  75                  80

Lys Ala Glu Ile Ala Gly
                85
```

<210> SEQ ID NO 14
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 14

```
Leu Gly Ile Asp Ser
1               5
```

<210> SEQ ID NO 15
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 15

```
Ala Pro Ala Pro Val Lys Ala Ala Ala Pro Ala Ala Pro Val Ala Ser
1               5                   10                  15

Ala Pro Ala Pro Ala
            20
```

<210> SEQ ID NO 16
<211> LENGTH: 3006
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 16

```
gcccccgccc cggtcaaggc tgctgcgcct gccgcccccg ttgcctcggc ccctgccccg      60

gctgtctcga acgagcttct tgagaaggcc gagactgtcg tcatggaggt cctcgccgcc     120

aagaccggct acgagaccga catgatcgag gctgacatgg agctcgagac cgagctcggc     180

attgactcca tcaagcgtgt cgagatcctc tccgaggtcc aggccatgct caatgtcgag     240

gccaaggatg tcgatgccct cagccgcact cgcactgttg gtgaggttgt caacgccatg     300

aaggccgaga tcgctggcag ctctgccccg gcgcctgctg ccgctgctcc ggctccggcc     360

aaggctgccc ctgccgccgc tgcgcctgct gtctcgaacg agcttctcga gaaggccgag     420

accgtcgtca tggaggtcct cgccgccaag actggctacg agactgacat gatcgagtcc     480

gacatggagc tcgagactga gctcggcatt gactccatca agcgtgtcga gatcctctcc     540

gaggttcagg ccatgctcaa cgtcgaggcc aaggacgtcg acgctctcag ccgcactcgc     600

actgtgggtg aggtcgtcaa cgccatgaag gctgagatcg ctggtggctc tgccccggcg     660

cctgccgccg ctgccccagg tccggctgct gccgcccctg cgcctgccgc cgccgcccct     720

gctgtctcga acgagcttct tgagaaggcc gagaccgtcg tcatggaggt cctcgccgcc     780
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aagactggct acgagactga catgatcgag tccgacatgg agctcgagac cgagctcggc    840
attgactcca tcaagcgtgt cgagattctc tccgaggtcc aggccatgct caacgtcgag    900
gccaaggacg tcgacgctct cagccgcacc cgcactgttg gcgaggtcgt cgatgccatg    960
aaggccgaga tcgctggtgg ctctgccccg gcgcctgccg ccgctgctcc tgctccggct   1020
gctgccgccc ctgcgcctgc cgcccctgcg cctgctgtct cgagcgagct tctcgagaag   1080
gccgagactg tcgtcatgga ggtcctcgcc gccaagactg gctacgagac tgacatgatc   1140
gagtccgaca tggagctcga gaccgagctc ggcattgact ccatcaagcg tgtcgagatt   1200
ctctccgagg tccaggccat gctcaacgtc gaggccaagg acgtcgacgc tctcagccgc   1260
acccgcactg ttggcgaggt cgtcgatgcc atgaaggccg agatcgctgg tggctctgcc   1320
ccggcgcctg ccgccgctgc tcctgctccg gctgctgccg cccctgcgcc tgccgcccct   1380
gcgcctgccg cccctgcgcc tgctgtctcg agcgagcttc tcgagaaggc cgagactgtc   1440
gtcatggagg tcctcgccgc caagactggc tacgagactg acatgattga gtccgacatg   1500
gagctcgaga ccgagctcgg cattgactcc atcaagcgtg tcgagattct ctccgaggtt   1560
caggccatgc tcaacgtcga ggccaaggac gtcgacgctc tcagccgcac tcgcactgtt   1620
ggtgaggtcg tcgatgccat gaaggctgag atcgctggca gctccgcctc ggcgcctgcc   1680
gccgctgctc ctgctccggc tgctgccgct cctgcgcccg ctgccgccgc ccctgctgtc   1740
tcgaacgagc ttctcgagaa agccgagact gtcgtcatgg aggtcctcgc cgccaagact   1800
ggctacgaga ctgacatgat cgagtccgac atggagctcg agactgagct cggcattgac   1860
tccatcaagc gtgtcgagat cctctccgag gttcaggcca tgctcaacgt cgaggccaag   1920
gacgtcgatg ccctcagccg cacccgcact gttggcgagg ttgtcgatgc catgaaggcc   1980
gagatcgctg gtggctctgc cccggcgcct gccgccgctg cccctgctcc ggctgccgcc   2040
gcccctgctg tctcgaacga gcttctcgag aaggccgaga ctgtcgtcat ggaggtcctc   2100
gccgccaaga ctggctacga gaccgacatg atcgagtccg acatggagct cgagaccgag   2160
ctcggcattg actccatcaa gcgtgtcgag attctctccg aggttcaggc catgctcaac   2220
gtcgaggcca aggacgtcga tgctctcagc cgcactcgca ctgttggcga ggtcgtcgat   2280
gccatgaagg ctgagatcgc cggcagctcc gccccggcgc ctgccgccgc tgctcctgct   2340
ccggctgctg ccgctcctgc gcccgctgcc gctgcccctg ctgtctcgag cgagcttctc   2400
gagaaggccg agaccgtcgt catggaggtc ctcgccgcca agactggcta cgagactgac   2460
atgattgagt ccgacatgga gctcgagact gagctcggca ttgactccat caagcgtgtc   2520
gagatcctct ccgaggttca ggccatgctc aacgtcgagg ccaaggacgt cgatgccctc   2580
agccgcaccc gcactgttgg cgaggttgtc gatgccatga aggccgagat cgctggtggc   2640
tctgccccgg cgcctgccgc cgctgcccct gctccggctg ccgccgcccc tgctgtctcg   2700
aacgagcttc ttgagaaggc cgagaccgtc gtcatggagg tcctcgccgc caagactggc   2760
tacgagaccg acatgatcga gtccgacatg gagctcgaga ccgagctcgg cattgactcc   2820
atcaagcgtg tcgagattct ctccgaggtt caggccatgc tcaacgtcga ggccaaggac   2880
gtcgacgctc tcagccgcac tcgcactgtt ggcgaggtcg tcgatgccat gaaggctgag   2940
atcgctggtg gctctgcccc ggcgcctgcc gccgctgctc ctgcctcggc tggcgccgcg   3000
cctgcg                                                              3006
```

<210> SEQ ID NO 17

```
<211> LENGTH: 2133
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(2133)

<400> SEQUENCE: 17

ttt ggc gct ctc ggc ggc ttc atc tcg cag cag gcg gag cgc ttc gag      48
Phe Gly Ala Leu Gly Gly Phe Ile Ser Gln Gln Ala Glu Arg Phe Glu
1               5                   10                  15

ccc gcc gaa atc ctc ggc ttc acg ctc atg tgc gcc aag ttc gcc aag      96
Pro Ala Glu Ile Leu Gly Phe Thr Leu Met Cys Ala Lys Phe Ala Lys
            20                  25                  30

gct tcc ctc tgc acg gct gtg gct ggc ggc cgc ccg gcc ttt atc ggt     144
Ala Ser Leu Cys Thr Ala Val Ala Gly Gly Arg Pro Ala Phe Ile Gly
        35                  40                  45

gtg gcg cgc ctt gac ggc cgc ctc gga ttc act tcg cag ggc act tct     192
Val Ala Arg Leu Asp Gly Arg Leu Gly Phe Thr Ser Gln Gly Thr Ser
    50                  55                  60

gac gcg ctc aag cgt gcc cag cgt ggt gcc atc ttt ggc ctc tgc aag     240
Asp Ala Leu Lys Arg Ala Gln Arg Gly Ala Ile Phe Gly Leu Cys Lys
65                  70                  75                  80

acc atc ggc ctc gag tgg tcc gag tct gac gtc ttt tcc cgc ggc gtg     288
Thr Ile Gly Leu Glu Trp Ser Glu Ser Asp Val Phe Ser Arg Gly Val
                85                  90                  95

gac att gct cag ggc atg cac ccc gag gat gcc gcc gtg gcg att gtg     336
Asp Ile Ala Gln Gly Met His Pro Glu Asp Ala Ala Val Ala Ile Val
            100                 105                 110

cgc gag atg gcg tgc gct gac att cgc att cgc gag gtc ggc att ggc     384
Arg Glu Met Ala Cys Ala Asp Ile Arg Ile Arg Glu Val Gly Ile Gly
        115                 120                 125

gca aac cag cag cgc tgc acg atc cgt gcc gcc aag ctc gag acc ggc     432
Ala Asn Gln Gln Arg Cys Thr Ile Arg Ala Ala Lys Leu Glu Thr Gly
    130                 135                 140

aac ccg cag cgc cag atc gcc aag gac gac gtg ctg ctc gtt tct ggc     480
Asn Pro Gln Arg Gln Ile Ala Lys Asp Asp Val Leu Leu Val Ser Gly
145                 150                 155                 160

ggc gct cgc ggc atc acg cct ctt tgc atc cgg gag atc acg cgc cag     528
Gly Ala Arg Gly Ile Thr Pro Leu Cys Ile Arg Glu Ile Thr Arg Gln
                165                 170                 175

atc gcg ggc ggc aag tac att ctg ctt ggc cgc agc aag gtc tct gcg     576
Ile Ala Gly Gly Lys Tyr Ile Leu Leu Gly Arg Ser Lys Val Ser Ala
            180                 185                 190

agc gaa ccg gca tgg tgc gct ggc atc act gac gag aag gct gtg caa     624
Ser Glu Pro Ala Trp Cys Ala Gly Ile Thr Asp Glu Lys Ala Val Gln
        195                 200                 205

aag gct gct acc cag gag ctc aag cgc gcc ttt agc gct ggc gag ggc     672
Lys Ala Ala Thr Gln Glu Leu Lys Arg Ala Phe Ser Ala Gly Glu Gly
    210                 215                 220

ccc aag ccc acg ccc cgc gct gtc act aag ctt gtg ggc tct gtt ctt     720
Pro Lys Pro Thr Pro Arg Ala Val Thr Lys Leu Val Gly Ser Val Leu
225                 230                 235                 240

ggc gct cgc gag gtg cgc agc tct att gct gcg att gaa gcg ctc ggc     768
Gly Ala Arg Glu Val Arg Ser Ser Ile Ala Ala Ile Glu Ala Leu Gly
                245                 250                 255

ggc aag gcc atc tac tcg tcg tgc gac gtg aac tct gcc gcc gac gtg     816
Gly Lys Ala Ile Tyr Ser Ser Cys Asp Val Asn Ser Ala Ala Asp Val
            260                 265                 270

gcc aag gcc gtg cgc gat gcc gag tcc cag ctc ggt gcc cgc gtc tcg     864
Ala Lys Ala Val Arg Asp Ala Glu Ser Gln Leu Gly Ala Arg Val Ser
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        275                 280                 285

ggc atc gtt cat gcc tcg ggc gtg ctc cgc gac cgt ctc atc gag aag      912
Gly Ile Val His Ala Ser Gly Val Leu Arg Asp Arg Leu Ile Glu Lys
    290                 295                 300

aag ctc ccc gac gag ttc gac gcc gtc ttt ggc acc aag gtc acc ggt      960
Lys Leu Pro Asp Glu Phe Asp Ala Val Phe Gly Thr Lys Val Thr Gly
305                 310                 315                 320

ctc gag aac ctc ctc gcc gcc gtc gac cgc gcc aac ctc aag cac atg     1008
Leu Glu Asn Leu Leu Ala Ala Val Asp Arg Ala Asn Leu Lys His Met
                325                 330                 335

gtc ctc ttc agc tcg ctc gcc ggc ttc cac ggc aac gtc ggc cag tct     1056
Val Leu Phe Ser Ser Leu Ala Gly Phe His Gly Asn Val Gly Gln Ser
            340                 345                 350

gac tac gcc atg gcc aac gag gcc ctt aac aag atg ggc ctc gag ctc     1104
Asp Tyr Ala Met Ala Asn Glu Ala Leu Asn Lys Met Gly Leu Glu Leu
        355                 360                 365

gcc aag gac gtc tcg gtc aag tcg atc tgc ttc ggt ccc tgg gac ggt     1152
Ala Lys Asp Val Ser Val Lys Ser Ile Cys Phe Gly Pro Trp Asp Gly
    370                 375                 380

ggc atg gtg acg ccg cag ctc aag aag cag ttc cag gag atg ggc gtg     1200
Gly Met Val Thr Pro Gln Leu Lys Lys Gln Phe Gln Glu Met Gly Val
385                 390                 395                 400

cag atc atc ccc cgc gag ggc ggc gct gat acc gtg gcg cgc atc gtg     1248
Gln Ile Ile Pro Arg Glu Gly Gly Ala Asp Thr Val Ala Arg Ile Val
                405                 410                 415

ctc ggc tcc tcg ccg gct gag atc ctt gtc ggc aac tgg cgc acc ccg     1296
Leu Gly Ser Ser Pro Ala Glu Ile Leu Val Gly Asn Trp Arg Thr Pro
            420                 425                 430

tcc aag aag gtc ggc tcg gac acc atc acc ctg cac cgc aag att tcc     1344
Ser Lys Lys Val Gly Ser Asp Thr Ile Thr Leu His Arg Lys Ile Ser
        435                 440                 445

gcc aag tcc aac ccc ttc ctc gag gac cac gtc atc cag ggc cgc cgc     1392
Ala Lys Ser Asn Pro Phe Leu Glu Asp His Val Ile Gln Gly Arg Arg
    450                 455                 460

gtg ctg ccc atg acg ctg gcc att ggc tcg ctc gcg gag acc tgc ctc     1440
Val Leu Pro Met Thr Leu Ala Ile Gly Ser Leu Ala Glu Thr Cys Leu
465                 470                 475                 480

ggc ctc ttc ccc ggc tac tcg ctc tgg gcc att gac gac gcc cag ctc     1488
Gly Leu Phe Pro Gly Tyr Ser Leu Trp Ala Ile Asp Asp Ala Gln Leu
                485                 490                 495

ttc aag ggt gtc act gtc gac ggc gac gtc aac tgc gag gtg acc ctc     1536
Phe Lys Gly Val Thr Val Asp Gly Asp Val Asn Cys Glu Val Thr Leu
            500                 505                 510

acc ccg tcg acg gcg ccc tcg ggc cgc gtc aac gtc cag gcc acg ctc     1584
Thr Pro Ser Thr Ala Pro Ser Gly Arg Val Asn Val Gln Ala Thr Leu
        515                 520                 525

aag acc ttt tcc agc ggc aag ctg gtc ccg gcc tac cgc gcc gtc atc     1632
Lys Thr Phe Ser Ser Gly Lys Leu Val Pro Ala Tyr Arg Ala Val Ile
    530                 535                 540

gtg ctc tcc aac cag ggc gcg ccc ccg gcc aac gcc acc atg cag ccg     1680
Val Leu Ser Asn Gln Gly Ala Pro Pro Ala Asn Ala Thr Met Gln Pro
545                 550                 555                 560

ccc tcg ctc gat gcc gat ccg gcg ctc cag ggc tcc gtc tac gac ggc     1728
Pro Ser Leu Asp Ala Asp Pro Ala Leu Gln Gly Ser Val Tyr Asp Gly
                565                 570                 575

aag acc ctc ttc cac ggc ccg gcc ttc cgc ggc atc gat gac gtg ctc     1776
Lys Thr Leu Phe His Gly Pro Ala Phe Arg Gly Ile Asp Asp Val Leu
            580                 585                 590

tcg tgc acc aag agc cag ctt gtg gcc aag tgc agc gct gtc ccc ggc     1824
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Ser Cys Thr Lys Ser Gln Leu Val Ala Lys Cys Ser Ala Val Pro Gly
        595                 600                 605

tcc gac gcc gct cgc ggc gag ttt gcc acg gac act gac gcc cat gac      1872
Ser Asp Ala Ala Arg Gly Glu Phe Ala Thr Asp Thr Asp Ala His Asp
    610                 615                 620

ccc ttc gtg aac gac ctg gcc ttt cag gcc atg ctc gtc tgg gtg cgc      1920
Pro Phe Val Asn Asp Leu Ala Phe Gln Ala Met Leu Val Trp Val Arg
625                 630                 635                 640

cgc acg ctc ggc cag gct gcg ctc ccc aac tcg atc cag cgc atc gtc      1968
Arg Thr Leu Gly Gln Ala Ala Leu Pro Asn Ser Ile Gln Arg Ile Val
                645                 650                 655

cag cac cgc ccg gtc ccg cag gac aag ccc ttc tac att acc ctc cgc      2016
Gln His Arg Pro Val Pro Gln Asp Lys Pro Phe Tyr Ile Thr Leu Arg
            660                 665                 670

tcc aac cag tcg ggc ggt cac tcc cag cac aag cac gcc ctt cag ttc      2064
Ser Asn Gln Ser Gly Gly His Ser Gln His Lys His Ala Leu Gln Phe
        675                 680                 685

cac aac gag cag ggc gat ctc ttc att gat gtc cag gct tcg gtc atc      2112
His Asn Glu Gln Gly Asp Leu Phe Ile Asp Val Gln Ala Ser Val Ile
    690                 695                 700

gcc acg gac agc ctt gcc ttc                                          2133
Ala Thr Asp Ser Leu Ala Phe
705                 710


<210> SEQ ID NO 18
<211> LENGTH: 711
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 18

Phe Gly Ala Leu Gly Gly Phe Ile Ser Gln Gln Ala Glu Arg Phe Glu
1               5                   10                  15

Pro Ala Glu Ile Leu Gly Phe Thr Leu Met Cys Ala Lys Phe Ala Lys
            20                  25                  30

Ala Ser Leu Cys Thr Ala Val Ala Gly Gly Arg Pro Ala Phe Ile Gly
        35                  40                  45

Val Ala Arg Leu Asp Gly Arg Leu Gly Phe Thr Ser Gln Gly Thr Ser
    50                  55                  60

Asp Ala Leu Lys Arg Ala Gln Arg Gly Ala Ile Phe Gly Leu Cys Lys
65                  70                  75                  80

Thr Ile Gly Leu Glu Trp Ser Glu Ser Asp Val Phe Ser Arg Gly Val
                85                  90                  95

Asp Ile Ala Gln Gly Met His Pro Glu Asp Ala Ala Val Ala Ile Val
            100                 105                 110

Arg Glu Met Ala Cys Ala Asp Ile Arg Ile Arg Glu Val Gly Ile Gly
        115                 120                 125

Ala Asn Gln Gln Arg Cys Thr Ile Arg Ala Ala Lys Leu Glu Thr Gly
    130                 135                 140

Asn Pro Gln Arg Gln Ile Ala Lys Asp Asp Val Leu Leu Val Ser Gly
145                 150                 155                 160

Gly Ala Arg Gly Ile Thr Pro Leu Cys Ile Arg Glu Ile Thr Arg Gln
                165                 170                 175

Ile Ala Gly Gly Lys Tyr Ile Leu Leu Gly Arg Ser Lys Val Ser Ala
            180                 185                 190

Ser Glu Pro Ala Trp Cys Ala Gly Ile Thr Asp Glu Lys Ala Val Gln
        195                 200                 205

Lys Ala Ala Thr Gln Glu Leu Lys Arg Ala Phe Ser Ala Gly Glu Gly
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    210                 215                 220

Pro Lys Pro Thr Pro Arg Ala Val Thr Lys Leu Val Gly Ser Val Leu
225                 230                 235                 240

Gly Ala Arg Glu Val Arg Ser Ser Ile Ala Ala Ile Glu Ala Leu Gly
                245                 250                 255

Gly Lys Ala Ile Tyr Ser Ser Cys Asp Val Asn Ser Ala Ala Asp Val
            260                 265                 270

Ala Lys Ala Val Arg Asp Ala Glu Ser Gln Leu Gly Ala Arg Val Ser
        275                 280                 285

Gly Ile Val His Ala Ser Gly Val Leu Arg Asp Arg Leu Ile Glu Lys
    290                 295                 300

Lys Leu Pro Asp Glu Phe Asp Ala Val Phe Gly Thr Lys Val Thr Gly
305                 310                 315                 320

Leu Glu Asn Leu Leu Ala Ala Val Asp Arg Ala Asn Leu Lys His Met
                325                 330                 335

Val Leu Phe Ser Ser Leu Ala Gly Phe His Gly Asn Val Gly Gln Ser
            340                 345                 350

Asp Tyr Ala Met Ala Asn Glu Ala Leu Asn Lys Met Gly Leu Glu Leu
        355                 360                 365

Ala Lys Asp Val Ser Val Lys Ser Ile Cys Phe Gly Pro Trp Asp Gly
    370                 375                 380

Gly Met Val Thr Pro Gln Leu Lys Lys Gln Phe Gln Glu Met Gly Val
385                 390                 395                 400

Gln Ile Ile Pro Arg Glu Gly Gly Ala Asp Thr Val Ala Arg Ile Val
                405                 410                 415

Leu Gly Ser Ser Pro Ala Glu Ile Leu Val Gly Asn Trp Arg Thr Pro
            420                 425                 430

Ser Lys Lys Val Gly Ser Asp Thr Ile Thr Leu His Arg Lys Ile Ser
        435                 440                 445

Ala Lys Ser Asn Pro Phe Leu Glu Asp His Val Ile Gln Gly Arg Arg
    450                 455                 460

Val Leu Pro Met Thr Leu Ala Ile Gly Ser Leu Ala Glu Thr Cys Leu
465                 470                 475                 480

Gly Leu Phe Pro Gly Tyr Ser Leu Trp Ala Ile Asp Asp Ala Gln Leu
                485                 490                 495

Phe Lys Gly Val Thr Val Asp Gly Asp Val Asn Cys Glu Val Thr Leu
            500                 505                 510

Thr Pro Ser Thr Ala Pro Ser Gly Arg Val Asn Val Gln Ala Thr Leu
        515                 520                 525

Lys Thr Phe Ser Ser Gly Lys Leu Val Pro Ala Tyr Arg Ala Val Ile
    530                 535                 540

Val Leu Ser Asn Gln Gly Ala Pro Pro Ala Asn Ala Thr Met Gln Pro
545                 550                 555                 560

Pro Ser Leu Asp Ala Asp Pro Ala Leu Gln Gly Ser Val Tyr Asp Gly
                565                 570                 575

Lys Thr Leu Phe His Gly Pro Ala Phe Arg Gly Ile Asp Asp Val Leu
            580                 585                 590

Ser Cys Thr Lys Ser Gln Leu Val Ala Lys Cys Ser Ala Val Pro Gly
        595                 600                 605

Ser Asp Ala Ala Arg Gly Glu Phe Ala Thr Asp Thr Asp Ala His Asp
    610                 615                 620

Pro Phe Val Asn Asp Leu Ala Phe Gln Ala Met Leu Val Trp Val Arg
625                 630                 635                 640
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Arg Thr Leu Gly Gln Ala Ala Leu Pro Asn Ser Ile Gln Arg Ile Val
                645                 650                 655

Gln His Arg Pro Val Pro Gln Asp Lys Pro Phe Tyr Ile Thr Leu Arg
            660                 665                 670

Ser Asn Gln Ser Gly Gly His Ser Gln His Lys His Ala Leu Gln Phe
        675                 680                 685

His Asn Glu Gln Gly Asp Leu Phe Ile Asp Val Gln Ala Ser Val Ile
    690                 695                 700

Ala Thr Asp Ser Leu Ala Phe
705                 710


<210> SEQ ID NO 19
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1350)

<400> SEQUENCE: 19

atg gcc gct cgg aat gtg agc gcc gcg cat gag atg cac gat gaa aag       48
Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

cgc atc gcc gtc gtc ggc atg gcc gtc cag tac gcc gga tgc aaa acc       96
Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

aag gac gag ttc tgg gag gtg ctc atg aac ggc aag gtc gag tcc aag      144
Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

gtg atc agc gac aaa cga ctc ggc tcc aac tac cgc gcc gag cac tac      192
Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

aaa gca gag cgc agc aag tat gcc gac acc ttt tgc aac gaa acg tac      240
Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80

ggc acc ctt gac gag aac gag atc gac aac gag cac gaa ctc ctc ctc      288
Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95

aac ctc gcc aag cag gca ctc gca gag aca tcc gtc aaa gac tcg aca      336
Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

cgc tgc ggc atc gtc agc ggc tgc ctc tcg ttc ccc atg gac aac ctc      384
Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

cag ggt gaa ctc ctc aac gtg tac caa aac cat gtc gag aaa aag ctc      432
Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

ggg gcc cgc gtc ttc aag gac gcc tcc cat tgg tcc gaa cgc gag cag      480
Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

tcc aac aaa ccc gag gcc ggt gac cgc cgc atc ttc atg gac ccg gcc      528
Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

tcc ttc gtc gcc gaa gaa ctc aac ctc ggc gcc ctt cac tac tcc gtc      576
Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

gac gca gca tgc gcc acg gcg ctc tac gtg ctc cgc ctc gcg cag gat      624
Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205
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cat ctc gtc tcc ggc gcc gcc gac gtc atg ctc tgc ggt gcc acc tgc      672
His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

ctg ccg gag ccc ttt ttc atc ctt tcg ggc ttt tcc acc ttc cag gcc      720
Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

atg ccc gtc ggc acg ggc cag aac gtg tcc atg ccg ctg cac aag gac      768
Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

agc cag ggc ctc acc ccg ggt gag ggc ggc tcc atc atg gtc ctc aag      816
Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270

cgt ctc gat gat gcc atc cgc gac ggc gac cac att tac ggc acc ctt      864
Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

ctc ggc gcc aat gtc agc aac tcc ggc aca ggt ctg ccc ctc aag ccc      912
Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

ctt ctc ccc agc gag aaa aag tgc ctc atg gac acc tac acg cgc att      960
Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

aac gtg cac ccg cac aag att cag tac gtc gag tgc cac gcc acc ggc     1008
Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

acg ccc cag ggt gat cgt gtg gaa atc gac gcc gtc aag gcc tgc ttt     1056
Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

gaa ggc aag gtc ccc cgt ttc ggt acc aca aag ggc aac ttt gga cac     1104
Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

acc cts gyc gca gcc ggc ttt gcc ggt atg tgc aag gtc ctc ctc tcc     1152
Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380

atg aag cat ggc atc atc ccg ccc acc ccg ggt atc gat gac gag acc     1200
Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

aag atg gac cct ctc gtc gtc tcc ggt gag gcc atc cca tgg cca gag     1248
Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415

acc aac ggc gag ccc aag cgc gcc ggt ctc tcg gcc ttt ggc ttt ggt     1296
Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

ggc acc aac gcc cat gcc gtc ttt gag gag cat gac ccc tcc aac gcc     1344
Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

gcc tgc                                                             1350
Ala Cys
    450


<210> SEQ ID NO 20
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (370)..(370)
<223> OTHER INFORMATION: The 'Xaa' at location 370 stands for Leu.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (371)..(371)
<223> OTHER INFORMATION: The 'Xaa' at location 371 stands for Ala, or
      Val.
```

<400> SEQUENCE: 20

```
Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80

Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95

Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205

His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270

Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380

Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415
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Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

Ala Cys
    450


<210> SEQ ID NO 21
<211> LENGTH: 1323
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1323)

<400> SEQUENCE: 21

tcg gcc cgc tgc ggc ggt gaa agc aac atg cgc atc gcc atc act ggt       48
Ser Ala Arg Cys Gly Gly Glu Ser Asn Met Arg Ile Ala Ile Thr Gly
1               5                   10                  15

atg gac gcc acc ttt ggc gct ctc aag gga ctc gac gcc ttc gag cgc       96
Met Asp Ala Thr Phe Gly Ala Leu Lys Gly Leu Asp Ala Phe Glu Arg
            20                  25                  30

gcc att tac acc ggc gct cac ggt gcc atc cca ctc cca gaa aag cgc      144
Ala Ile Tyr Thr Gly Ala His Gly Ala Ile Pro Leu Pro Glu Lys Arg
        35                  40                  45

tgg cgc ttt ctc ggc aag gac aag gac ttt ctt gac ctc tgc ggc gtc      192
Trp Arg Phe Leu Gly Lys Asp Lys Asp Phe Leu Asp Leu Cys Gly Val
    50                  55                  60

aag gcc acc ccg cac ggc tgc tac att gaa gat gtt gag gtc gac ttc      240
Lys Ala Thr Pro His Gly Cys Tyr Ile Glu Asp Val Glu Val Asp Phe
65                  70                  75                  80

cag cgc ctc cgc acg ccc atg acc cct gaa gac atg ctc ctc cct cag      288
Gln Arg Leu Arg Thr Pro Met Thr Pro Glu Asp Met Leu Leu Pro Gln
                85                  90                  95

cag ctt ctg gcc gtc acc acc att gac cgc gcc atc ctc gac tcg gga      336
Gln Leu Leu Ala Val Thr Thr Ile Asp Arg Ala Ile Leu Asp Ser Gly
            100                 105                 110

atg aaa aag ggt ggc aat gtc gcc gtc ttt gtc ggc ctc ggc acc gac      384
Met Lys Lys Gly Gly Asn Val Ala Val Phe Val Gly Leu Gly Thr Asp
        115                 120                 125

ctc gag ctc tac cgt cac cgt gct cgc gtc gct ctc aag gag cgc gtc      432
Leu Glu Leu Tyr Arg His Arg Ala Arg Val Ala Leu Lys Glu Arg Val
    130                 135                 140

cgc cct gaa gcc tcc aag aag ctc aat gac atg atg cag tac att aac      480
Arg Pro Glu Ala Ser Lys Lys Leu Asn Asp Met Met Gln Tyr Ile Asn
145                 150                 155                 160

gac tgc ggc aca tcc aca tcg tac acc tcg tac att ggc aac ctc gtc      528
Asp Cys Gly Thr Ser Thr Ser Tyr Thr Ser Tyr Ile Gly Asn Leu Val
                165                 170                 175

gcc acg cgc gtc tcg tcg cag tgg ggc ttc acg ggc ccc tcc ttt acg      576
Ala Thr Arg Val Ser Ser Gln Trp Gly Phe Thr Gly Pro Ser Phe Thr
            180                 185                 190

atc acc gag ggc aac aac tcc gtc tac cgc tgc gcc gag ctc ggc aag      624
Ile Thr Glu Gly Asn Asn Ser Val Tyr Arg Cys Ala Glu Leu Gly Lys
        195                 200                 205

tac ctc ctc gag acc ggc gag gtc gat ggc gtc gtc gtt gcg ggt gtc      672
Tyr Leu Leu Glu Thr Gly Glu Val Asp Gly Val Val Val Ala Gly Val
    210                 215                 220

gat ctc tgc ggc agt gcc gaa aac ctt tac gtc aag tct cgc cgc ttc      720
Asp Leu Cys Gly Ser Ala Glu Asn Leu Tyr Val Lys Ser Arg Arg Phe
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225                 230                 235                 240

aag gtg tcc acc tcc gat acc ccg cgc gcc agc ttt gac gcc gcc gcc       768
Lys Val Ser Thr Ser Asp Thr Pro Arg Ala Ser Phe Asp Ala Ala Ala
                245                 250                 255

gat ggc tac ttt gtc ggc gag ggc tgc ggt gcc ttt gtg ctc aag cgt       816
Asp Gly Tyr Phe Val Gly Glu Gly Cys Gly Ala Phe Val Leu Lys Arg
            260                 265                 270

gag act agc tgc acc aag gac gac cgt atc tac gct tgc atg gat gcc       864
Glu Thr Ser Cys Thr Lys Asp Asp Arg Ile Tyr Ala Cys Met Asp Ala
        275                 280                 285

atc gtc cct ggc aac gtc cct agc gcc tgc ttg cgc gag gcc ctc gac       912
Ile Val Pro Gly Asn Val Pro Ser Ala Cys Leu Arg Glu Ala Leu Asp
    290                 295                 300

cag gcg cgc gtc aag ccg ggc gat atc gag atg ctc gag ctc agc gcc       960
Gln Ala Arg Val Lys Pro Gly Asp Ile Glu Met Leu Glu Leu Ser Ala
305                 310                 315                 320

gac tcc gcc cgc cac ctc aag gac ccg tcc gtc ctg ccc aag gag ctc      1008
Asp Ser Ala Arg His Leu Lys Asp Pro Ser Val Leu Pro Lys Glu Leu
                325                 330                 335

act gcc gag gag gaa atc ggc ggc ctt cag acg atc ctt cgt gac gat      1056
Thr Ala Glu Glu Glu Ile Gly Gly Leu Gln Thr Ile Leu Arg Asp Asp
            340                 345                 350

gac aag ctc ccg cgc aac gtc gca acg ggc agt gtc aag gcc acc gtc      1104
Asp Lys Leu Pro Arg Asn Val Ala Thr Gly Ser Val Lys Ala Thr Val
        355                 360                 365

ggt gac acc ggt tat gcc tct ggt gct gcc agc ctc atc aag gct gcg      1152
Gly Asp Thr Gly Tyr Ala Ser Gly Ala Ala Ser Leu Ile Lys Ala Ala
    370                 375                 380

ctt tgc atc tac aac cgc tac ctg ccc agc aac ggc gac gac tgg gat      1200
Leu Cys Ile Tyr Asn Arg Tyr Leu Pro Ser Asn Gly Asp Asp Trp Asp
385                 390                 395                 400

gaa ccc gcc cct gag gcg ccc tgg gac agc acc ctc ttt gcg tgc cag      1248
Glu Pro Ala Pro Glu Ala Pro Trp Asp Ser Thr Leu Phe Ala Cys Gln
                405                 410                 415

acc tcg cgc gct tgg ctc aag aac cct ggc gag cgt cgc tat gcg gcc      1296
Thr Ser Arg Ala Trp Leu Lys Asn Pro Gly Glu Arg Arg Tyr Ala Ala
            420                 425                 430

gtc tcg ggc gtc tcc gag acg cgc tcg                                  1323
Val Ser Gly Val Ser Glu Thr Arg Ser
        435                 440


<210> SEQ ID NO 22
<211> LENGTH: 441
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 22

Ser Ala Arg Cys Gly Gly Glu Ser Asn Met Arg Ile Ala Ile Thr Gly
1               5                   10                  15

Met Asp Ala Thr Phe Gly Ala Leu Lys Gly Leu Asp Ala Phe Glu Arg
            20                  25                  30

Ala Ile Tyr Thr Gly Ala His Gly Ala Ile Pro Leu Pro Glu Lys Arg
        35                  40                  45

Trp Arg Phe Leu Gly Lys Asp Lys Asp Phe Leu Asp Leu Cys Gly Val
    50                  55                  60

Lys Ala Thr Pro His Gly Cys Tyr Ile Glu Asp Val Glu Val Asp Phe
65                  70                  75                  80

Gln Arg Leu Arg Thr Pro Met Thr Pro Glu Asp Met Leu Leu Pro Gln
                85                  90                  95
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Gln Leu Leu Ala Val Thr Thr Ile Asp Arg Ala Ile Leu Asp Ser Gly
        100                 105                 110

Met Lys Lys Gly Gly Asn Val Ala Val Phe Val Gly Leu Gly Thr Asp
    115                 120                 125

Leu Glu Leu Tyr Arg His Arg Ala Arg Val Ala Leu Lys Glu Arg Val
    130                 135                 140

Arg Pro Glu Ala Ser Lys Lys Leu Asn Asp Met Met Gln Tyr Ile Asn
145                 150                 155                 160

Asp Cys Gly Thr Ser Thr Ser Tyr Thr Ser Tyr Ile Gly Asn Leu Val
                165                 170                 175

Ala Thr Arg Val Ser Ser Gln Trp Gly Phe Thr Gly Pro Ser Phe Thr
            180                 185                 190

Ile Thr Glu Gly Asn Asn Ser Val Tyr Arg Cys Ala Glu Leu Gly Lys
        195                 200                 205

Tyr Leu Leu Glu Thr Gly Glu Val Asp Gly Val Val Val Ala Gly Val
    210                 215                 220

Asp Leu Cys Gly Ser Ala Glu Asn Leu Tyr Val Lys Ser Arg Arg Phe
225                 230                 235                 240

Lys Val Ser Thr Ser Asp Thr Pro Arg Ala Ser Phe Asp Ala Ala Ala
                245                 250                 255

Asp Gly Tyr Phe Val Gly Glu Gly Cys Gly Ala Phe Val Leu Lys Arg
            260                 265                 270

Glu Thr Ser Cys Thr Lys Asp Asp Arg Ile Tyr Ala Cys Met Asp Ala
        275                 280                 285

Ile Val Pro Gly Asn Val Pro Ser Ala Cys Leu Arg Glu Ala Leu Asp
    290                 295                 300

Gln Ala Arg Val Lys Pro Gly Asp Ile Glu Met Leu Glu Leu Ser Ala
305                 310                 315                 320

Asp Ser Ala Arg His Leu Lys Asp Pro Ser Val Leu Pro Lys Glu Leu
                325                 330                 335

Thr Ala Glu Glu Glu Ile Gly Gly Leu Gln Thr Ile Leu Arg Asp Asp
            340                 345                 350

Asp Lys Leu Pro Arg Asn Val Ala Thr Gly Ser Val Lys Ala Thr Val
        355                 360                 365

Gly Asp Thr Gly Tyr Ala Ser Gly Ala Ala Ser Leu Ile Lys Ala Ala
    370                 375                 380

Leu Cys Ile Tyr Asn Arg Tyr Leu Pro Ser Asn Gly Asp Asp Trp Asp
385                 390                 395                 400

Glu Pro Ala Pro Glu Ala Pro Trp Asp Ser Thr Leu Phe Ala Cys Gln
                405                 410                 415

Thr Ser Arg Ala Trp Leu Lys Asn Pro Gly Glu Arg Arg Tyr Ala Ala
            420                 425                 430

Val Ser Gly Val Ser Glu Thr Arg Ser
        435                 440


<210> SEQ ID NO 23
<211> LENGTH: 1500
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1500)

<400> SEQUENCE: 23

tgc tat tcc gtg ctc ctc tcc gaa gcc gag ggc cac tac gag cgc gag       48
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Cys Tyr Ser Val Leu Leu Ser Glu Ala Glu Gly His Tyr Glu Arg Glu
1               5                   10                  15

aac cgc atc tcg ctc gac gag gag gcg ccc aag ctc att gtg ctt cgc       96
Asn Arg Ile Ser Leu Asp Glu Glu Ala Pro Lys Leu Ile Val Leu Arg
            20                  25                  30

gcc gac tcc cac gag gag atc ctt ggt cgc ctc gac aag atc cgc gag      144
Ala Asp Ser His Glu Glu Ile Leu Gly Arg Leu Asp Lys Ile Arg Glu
        35                  40                  45

cgc ttc ttg cag ccc acg ggc gcc gcc ccg cgc gag tcc gag ctc aag      192
Arg Phe Leu Gln Pro Thr Gly Ala Ala Pro Arg Glu Ser Glu Leu Lys
    50                  55                  60

gcg cag gcc cgc cgc atc ttc ctc gag ctc ctc ggc gag acc ctt gcc      240
Ala Gln Ala Arg Arg Ile Phe Leu Glu Leu Leu Gly Glu Thr Leu Ala
65                  70                  75                  80

cag gat gcc gct tct tca ggc tcg caa aag ccc ctc gct ctc agc ctc      288
Gln Asp Ala Ala Ser Ser Gly Ser Gln Lys Pro Leu Ala Leu Ser Leu
                85                  90                  95

gtc tcc acg ccc tcc aag ctc cag cgc gag gtc gag ctc gcg gcc aag      336
Val Ser Thr Pro Ser Lys Leu Gln Arg Glu Val Glu Leu Ala Ala Lys
            100                 105                 110

ggt atc ccg cgc tgc ctc aag atg cgc cgc gat tgg agc tcc cct gct      384
Gly Ile Pro Arg Cys Leu Lys Met Arg Arg Asp Trp Ser Ser Pro Ala
        115                 120                 125

ggc agc cgc tac gcg cct gag ccg ctc gcc agc gac cgc gtc gcc ttc      432
Gly Ser Arg Tyr Ala Pro Glu Pro Leu Ala Ser Asp Arg Val Ala Phe
    130                 135                 140

atg tac ggc gaa ggt cgc agc cct tac tac ggc atc acc caa gac att      480
Met Tyr Gly Glu Gly Arg Ser Pro Tyr Tyr Gly Ile Thr Gln Asp Ile
145                 150                 155                 160

cac cgc att tgg ccc gaa ctc cac gag gtc atc aac gaa aag acg aac      528
His Arg Ile Trp Pro Glu Leu His Glu Val Ile Asn Glu Lys Thr Asn
                165                 170                 175

cgt ctc tgg gcc gaa ggc gac cgc tgg gtc atg ccg cgc gcc agc ttc      576
Arg Leu Trp Ala Glu Gly Asp Arg Trp Val Met Pro Arg Ala Ser Phe
            180                 185                 190

aag tcg gag ctc gag agc cag cag caa gag ttt gat cgc aac atg att      624
Lys Ser Glu Leu Glu Ser Gln Gln Gln Glu Phe Asp Arg Asn Met Ile
        195                 200                 205

gaa atg ttc cgt ctt gga atc ctc acc tca att gcc ttc acc aat ctg      672
Glu Met Phe Arg Leu Gly Ile Leu Thr Ser Ile Ala Phe Thr Asn Leu
    210                 215                 220

gcg cgc gac gtt ctc aac atc acg ccc aag gcc gcc ttt ggc ctc agt      720
Ala Arg Asp Val Leu Asn Ile Thr Pro Lys Ala Ala Phe Gly Leu Ser
225                 230                 235                 240

ctt ggc gag att tcc atg att ttt gcc ttt tcc aag aag aac ggt ctc      768
Leu Gly Glu Ile Ser Met Ile Phe Ala Phe Ser Lys Lys Asn Gly Leu
                245                 250                 255

atc tcc gac cag ctc acc aag gat ctt cgc gag tcc gac gtg tgg aac      816
Ile Ser Asp Gln Leu Thr Lys Asp Leu Arg Glu Ser Asp Val Trp Asn
            260                 265                 270

aag gct ctg gcc gtt gaa ttt aat gcg ctg cgc gag gcc tgg ggc att      864
Lys Ala Leu Ala Val Glu Phe Asn Ala Leu Arg Glu Ala Trp Gly Ile
        275                 280                 285

cca cag agt gtc ccc aag gac gag ttc tgg caa ggc tac att gtg cgc      912
Pro Gln Ser Val Pro Lys Asp Glu Phe Trp Gln Gly Tyr Ile Val Arg
    290                 295                 300

ggc acc aag cag gat atc gag gcg gcc atc gcc ccg gac agc aag tac      960
Gly Thr Lys Gln Asp Ile Glu Ala Ala Ile Ala Pro Asp Ser Lys Tyr
305                 310                 315                 320
```

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gtg cgc ctc acc atc atc aat gat gcc aac acc gcc ctc att agc ggc      1008
Val Arg Leu Thr Ile Ile Asn Asp Ala Asn Thr Ala Leu Ile Ser Gly
                325                 330                 335

aag ccc gac gcc tgc aag gct gcg atc gcg cgt ctc ggt ggc aac att      1056
Lys Pro Asp Ala Cys Lys Ala Ala Ile Ala Arg Leu Gly Gly Asn Ile
            340                 345                 350

cct gcg ctt ccc gtg acc cag ggc atg tgc ggc cac tgc ccc gag gtg      1104
Pro Ala Leu Pro Val Thr Gln Gly Met Cys Gly His Cys Pro Glu Val
        355                 360                 365

gga cct tat acc aag gat atc gcc aag atc cat gcc aac ctt gag ttc      1152
Gly Pro Tyr Thr Lys Asp Ile Ala Lys Ile His Ala Asn Leu Glu Phe
    370                 375                 380

ccc gtt gtc gac ggc ctt gac ctc tgg acc aca atc aac cag aag cgc      1200
Pro Val Val Asp Gly Leu Asp Leu Trp Thr Thr Ile Asn Gln Lys Arg
385                 390                 395                 400

ctc gtg cca cgc gcc acg ggc gcc aag gac gaa tgg gcc cct tct tcc      1248
Leu Val Pro Arg Ala Thr Gly Ala Lys Asp Glu Trp Ala Pro Ser Ser
                405                 410                 415

ttt ggc gag tac gcc ggc cag ctc tac gag aag cag gct aac ttc ccc      1296
Phe Gly Glu Tyr Ala Gly Gln Leu Tyr Glu Lys Gln Ala Asn Phe Pro
            420                 425                 430

caa atc gtc gag acc att tac aag caa aac tac gac gtc ttt gtc gag      1344
Gln Ile Val Glu Thr Ile Tyr Lys Gln Asn Tyr Asp Val Phe Val Glu
        435                 440                 445

gtt ggg ccc aac aac cac cgt agc acc gca gtg cgc acc acg ctt ggt      1392
Val Gly Pro Asn Asn His Arg Ser Thr Ala Val Arg Thr Thr Leu Gly
    450                 455                 460

ccc cag cgc aac cac ctt gct ggc gcc atc gac aag cag aac gag gat      1440
Pro Gln Arg Asn His Leu Ala Gly Ala Ile Asp Lys Gln Asn Glu Asp
465                 470                 475                 480

gct tgg acg acc atc gtc aag ctt gtg gct tcg ctc aag gcc cac ctt      1488
Ala Trp Thr Thr Ile Val Lys Leu Val Ala Ser Leu Lys Ala His Leu
                485                 490                 495

gtt cct ggc gtc                                                      1500
Val Pro Gly Val
            500


<210> SEQ ID NO 24
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 24

Cys Tyr Ser Val Leu Leu Ser Glu Ala Glu Gly His Tyr Glu Arg Glu
1               5                   10                  15

Asn Arg Ile Ser Leu Asp Glu Glu Ala Pro Lys Leu Ile Val Leu Arg
            20                  25                  30

Ala Asp Ser His Glu Glu Ile Leu Gly Arg Leu Asp Lys Ile Arg Glu
        35                  40                  45

Arg Phe Leu Gln Pro Thr Gly Ala Ala Pro Arg Glu Ser Glu Leu Lys
    50                  55                  60

Ala Gln Ala Arg Arg Ile Phe Leu Glu Leu Leu Gly Glu Thr Leu Ala
65                  70                  75                  80

Gln Asp Ala Ala Ser Ser Gly Ser Gln Lys Pro Leu Ala Leu Ser Leu
                85                  90                  95

Val Ser Thr Pro Ser Lys Leu Gln Arg Glu Val Glu Leu Ala Ala Lys
            100                 105                 110

Gly Ile Pro Arg Cys Leu Lys Met Arg Arg Asp Trp Ser Ser Pro Ala
        115                 120                 125
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Gly Ser Arg Tyr Ala Pro Glu Pro Leu Ala Ser Asp Arg Val Ala Phe
    130                 135                 140

Met Tyr Gly Glu Gly Arg Ser Pro Tyr Tyr Gly Ile Thr Gln Asp Ile
145                 150                 155                 160

His Arg Ile Trp Pro Glu Leu His Glu Val Ile Asn Glu Lys Thr Asn
                165                 170                 175

Arg Leu Trp Ala Glu Gly Asp Arg Trp Val Met Pro Arg Ala Ser Phe
            180                 185                 190

Lys Ser Glu Leu Glu Ser Gln Gln Gln Glu Phe Asp Arg Asn Met Ile
        195                 200                 205

Glu Met Phe Arg Leu Gly Ile Leu Thr Ser Ile Ala Phe Thr Asn Leu
    210                 215                 220

Ala Arg Asp Val Leu Asn Ile Thr Pro Lys Ala Ala Phe Gly Leu Ser
225                 230                 235                 240

Leu Gly Glu Ile Ser Met Ile Phe Ala Phe Ser Lys Lys Asn Gly Leu
                245                 250                 255

Ile Ser Asp Gln Leu Thr Lys Asp Leu Arg Glu Ser Asp Val Trp Asn
            260                 265                 270

Lys Ala Leu Ala Val Glu Phe Asn Ala Leu Arg Glu Ala Trp Gly Ile
        275                 280                 285

Pro Gln Ser Val Pro Lys Asp Glu Phe Trp Gln Gly Tyr Ile Val Arg
    290                 295                 300

Gly Thr Lys Gln Asp Ile Glu Ala Ala Ile Ala Pro Asp Ser Lys Tyr
305                 310                 315                 320

Val Arg Leu Thr Ile Ile Asn Asp Ala Asn Thr Ala Leu Ile Ser Gly
                325                 330                 335

Lys Pro Asp Ala Cys Lys Ala Ala Ile Ala Arg Leu Gly Gly Asn Ile
            340                 345                 350

Pro Ala Leu Pro Val Thr Gln Gly Met Cys Gly His Cys Pro Glu Val
        355                 360                 365

Gly Pro Tyr Thr Lys Asp Ile Ala Lys Ile His Ala Asn Leu Glu Phe
    370                 375                 380

Pro Val Val Asp Gly Leu Asp Leu Trp Thr Thr Ile Asn Gln Lys Arg
385                 390                 395                 400

Leu Val Pro Arg Ala Thr Gly Ala Lys Asp Glu Trp Ala Pro Ser Ser
                405                 410                 415

Phe Gly Glu Tyr Ala Gly Gln Leu Tyr Glu Lys Gln Ala Asn Phe Pro
            420                 425                 430

Gln Ile Val Glu Thr Ile Tyr Lys Gln Asn Tyr Asp Val Phe Val Glu
        435                 440                 445

Val Gly Pro Asn Asn His Arg Ser Thr Ala Val Arg Thr Thr Leu Gly
    450                 455                 460

Pro Gln Arg Asn His Leu Ala Gly Ala Ile Asp Lys Gln Asn Glu Asp
465                 470                 475                 480

Ala Trp Thr Thr Ile Val Lys Leu Val Ala Ser Leu Lys Ala His Leu
                485                 490                 495

Val Pro Gly Val
            500


<210> SEQ ID NO 25
<211> LENGTH: 1530
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
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<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1530)

<400> SEQUENCE: 25

ctg ctc gat ctc gac agt atg ctt gcg ctg agc tct gcc agt gcc tcc       48
Leu Leu Asp Leu Asp Ser Met Leu Ala Leu Ser Ser Ala Ser Ala Ser
1               5                   10                  15

ggc aac ctt gtt gag act gcg cct agc gac gcc tcg gtc att gtg ccg       96
Gly Asn Leu Val Glu Thr Ala Pro Ser Asp Ala Ser Val Ile Val Pro
            20                  25                  30

ccc tgc aac att gcg gat ctc ggc agc cgc gcc ttc atg aaa acg tac      144
Pro Cys Asn Ile Ala Asp Leu Gly Ser Arg Ala Phe Met Lys Thr Tyr
        35                  40                  45

ggt gtt tcg gcg cct ctg tac acg ggc gcc atg gcc aag ggc att gcc      192
Gly Val Ser Ala Pro Leu Tyr Thr Gly Ala Met Ala Lys Gly Ile Ala
    50                  55                  60

tct gcg gac ctc gtc att gcc gcc ggc cgc cag ggc atc ctt gcg tcc      240
Ser Ala Asp Leu Val Ile Ala Ala Gly Arg Gln Gly Ile Leu Ala Ser
65                  70                  75                  80

ttt ggc gcc ggc gga ctt ccc atg cag gtt gtg cgt gag tcc atc gaa      288
Phe Gly Ala Gly Gly Leu Pro Met Gln Val Val Arg Glu Ser Ile Glu
                85                  90                  95

aag att cag gcc gcc ctg ccc aat ggc ccg tac gct gtc aac ctt atc      336
Lys Ile Gln Ala Ala Leu Pro Asn Gly Pro Tyr Ala Val Asn Leu Ile
            100                 105                 110

cat tct ccc ttt gac agc aac ctc gaa aag ggc aat gtc gat ctc ttc      384
His Ser Pro Phe Asp Ser Asn Leu Glu Lys Gly Asn Val Asp Leu Phe
        115                 120                 125

ctc gag aag ggt gtc acc ttt gtc gag gcc tcg gcc ttt atg acg ctc      432
Leu Glu Lys Gly Val Thr Phe Val Glu Ala Ser Ala Phe Met Thr Leu
    130                 135                 140

acc ccg cag gtc gtg cgg tac cgc gcg gct ggc ctc acg cgc aac gcc      480
Thr Pro Gln Val Val Arg Tyr Arg Ala Ala Gly Leu Thr Arg Asn Ala
145                 150                 155                 160

gac ggc tcg gtc aac atc cgc aac cgt atc att ggc aag gtc tcg cgc      528
Asp Gly Ser Val Asn Ile Arg Asn Arg Ile Ile Gly Lys Val Ser Arg
                165                 170                 175

acc gag ctc gcc gag atg ttc atg cgt cct gcg ccc gag cac ctt ctt      576
Thr Glu Leu Ala Glu Met Phe Met Arg Pro Ala Pro Glu His Leu Leu
            180                 185                 190

cag aag ctc att gct tcc ggc gag atc aac cag gag cag gcc gag ctc      624
Gln Lys Leu Ile Ala Ser Gly Glu Ile Asn Gln Glu Gln Ala Glu Leu
        195                 200                 205

gcc cgc cgt gtt ccc gtc gct gac gac atc gcg gtc gaa gct gac tcg      672
Ala Arg Arg Val Pro Val Ala Asp Asp Ile Ala Val Glu Ala Asp Ser
    210                 215                 220

ggt ggc cac acc gac aac cgc ccc atc cac gtc att ctg ccc ctc atc      720
Gly Gly His Thr Asp Asn Arg Pro Ile His Val Ile Leu Pro Leu Ile
225                 230                 235                 240

atc aac ctt cgc gac cgc ctt cac cgc gag tgc ggc tac ccg gcc aac      768
Ile Asn Leu Arg Asp Arg Leu His Arg Glu Cys Gly Tyr Pro Ala Asn
                245                 250                 255

ctt cgc gtc cgt gtg ggc gcc ggc ggt ggc att ggg tgc ccc cag gcg      816
Leu Arg Val Arg Val Gly Ala Gly Gly Gly Ile Gly Cys Pro Gln Ala
            260                 265                 270

gcg ctg gcc acc ttc aac atg ggt gcc tcc ttt att gtc acc ggc acc      864
Ala Leu Ala Thr Phe Asn Met Gly Ala Ser Phe Ile Val Thr Gly Thr
        275                 280                 285

gtg aac cag gtc gcc aag cag tcg ggc acg tgc gac aat gtg cgc aag      912
Val Asn Gln Val Ala Lys Gln Ser Gly Thr Cys Asp Asn Val Arg Lys
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    290                 295                 300

cag ctc gcg aag gcc act tac tcg gac gta tgc atg gcc ccg gct gcc      960
Gln Leu Ala Lys Ala Thr Tyr Ser Asp Val Cys Met Ala Pro Ala Ala
305                 310                 315                 320

gac atg ttc gag gaa ggc gtc aag ctt cag gtc ctc aag aag gga acc     1008
Asp Met Phe Glu Glu Gly Val Lys Leu Gln Val Leu Lys Lys Gly Thr
                325                 330                 335

atg ttt ccc tcg cgc gcc aac aag ctc tac gag ctc ttt tgc aag tac     1056
Met Phe Pro Ser Arg Ala Asn Lys Leu Tyr Glu Leu Phe Cys Lys Tyr
            340                 345                 350

gac tcg ttc gag tcc atg ccc ccc gca gag ctt gcg cgc gtc gag aag     1104
Asp Ser Phe Glu Ser Met Pro Pro Ala Glu Leu Ala Arg Val Glu Lys
        355                 360                 365

cgc atc ttc agc cgc gcg ctc gaa gag gtc tgg gac gag acc aaa aac     1152
Arg Ile Phe Ser Arg Ala Leu Glu Glu Val Trp Asp Glu Thr Lys Asn
    370                 375                 380

ttt tac att aac cgt ctt cac aac ccg gag aag atc cag cgc gcc gag     1200
Phe Tyr Ile Asn Arg Leu His Asn Pro Glu Lys Ile Gln Arg Ala Glu
385                 390                 395                 400

cgc gac ccc aag ctc aag atg tcg ctg tgc ttt cgc tgg tac ctg agc     1248
Arg Asp Pro Lys Leu Lys Met Ser Leu Cys Phe Arg Trp Tyr Leu Ser
                405                 410                 415

ctg gcg agc cgc tgg gcc aac act gga gct tcc gat cgc gtc atg gac     1296
Leu Ala Ser Arg Trp Ala Asn Thr Gly Ala Ser Asp Arg Val Met Asp
            420                 425                 430

tac cag gtc tgg tgc ggt cct gcc att ggt tcc ttc aac gat ttc atc     1344
Tyr Gln Val Trp Cys Gly Pro Ala Ile Gly Ser Phe Asn Asp Phe Ile
        435                 440                 445

aag gga act tac ctt gat ccg gcc gtc gca aac gag tac ccg tgc gtc     1392
Lys Gly Thr Tyr Leu Asp Pro Ala Val Ala Asn Glu Tyr Pro Cys Val
    450                 455                 460

gtt cag att aac aag cag atc ctt cgt gga gcg tgc ttc ttg cgc cgt     1440
Val Gln Ile Asn Lys Gln Ile Leu Arg Gly Ala Cys Phe Leu Arg Arg
465                 470                 475                 480

ctc gaa att ctg cgc aac gca cgc ctt tcc gat ggc gct gcc gct ctt     1488
Leu Glu Ile Leu Arg Asn Ala Arg Leu Ser Asp Gly Ala Ala Ala Leu
                485                 490                 495

gtg gcc agc atc gat gac aca tac gtc ccg gcc gag aag ctg             1530
Val Ala Ser Ile Asp Asp Thr Tyr Val Pro Ala Glu Lys Leu
            500                 505                 510


<210> SEQ ID NO 26
<211> LENGTH: 510
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 26

Leu Leu Asp Leu Asp Ser Met Leu Ala Leu Ser Ser Ala Ser Ala Ser
1               5                   10                  15

Gly Asn Leu Val Glu Thr Ala Pro Ser Asp Ala Ser Val Ile Val Pro
            20                  25                  30

Pro Cys Asn Ile Ala Asp Leu Gly Ser Arg Ala Phe Met Lys Thr Tyr
        35                  40                  45

Gly Val Ser Ala Pro Leu Tyr Thr Gly Ala Met Ala Lys Gly Ile Ala
    50                  55                  60

Ser Ala Asp Leu Val Ile Ala Ala Gly Arg Gln Gly Ile Leu Ala Ser
65                  70                  75                  80

Phe Gly Ala Gly Gly Leu Pro Met Gln Val Val Arg Glu Ser Ile Glu
                85                  90                  95
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Lys Ile Gln Ala Ala Leu Pro Asn Gly Pro Tyr Ala Val Asn Leu Ile
            100                 105                 110

His Ser Pro Phe Asp Ser Asn Leu Glu Lys Gly Asn Val Asp Leu Phe
        115                 120                 125

Leu Glu Lys Gly Val Thr Phe Val Glu Ala Ser Ala Phe Met Thr Leu
    130                 135                 140

Thr Pro Gln Val Val Arg Tyr Arg Ala Ala Gly Leu Thr Arg Asn Ala
145                 150                 155                 160

Asp Gly Ser Val Asn Ile Arg Asn Arg Ile Ile Gly Lys Val Ser Arg
                165                 170                 175

Thr Glu Leu Ala Glu Met Phe Met Arg Pro Ala Pro Glu His Leu Leu
            180                 185                 190

Gln Lys Leu Ile Ala Ser Gly Glu Ile Asn Gln Glu Gln Ala Glu Leu
        195                 200                 205

Ala Arg Arg Val Pro Val Ala Asp Asp Ile Ala Val Glu Ala Asp Ser
    210                 215                 220

Gly Gly His Thr Asp Asn Arg Pro Ile His Val Ile Leu Pro Leu Ile
225                 230                 235                 240

Ile Asn Leu Arg Asp Arg Leu His Arg Glu Cys Gly Tyr Pro Ala Asn
                245                 250                 255

Leu Arg Val Arg Val Gly Ala Gly Gly Gly Ile Gly Cys Pro Gln Ala
            260                 265                 270

Ala Leu Ala Thr Phe Asn Met Gly Ala Ser Phe Ile Val Thr Gly Thr
        275                 280                 285

Val Asn Gln Val Ala Lys Gln Ser Gly Thr Cys Asp Asn Val Arg Lys
    290                 295                 300

Gln Leu Ala Lys Ala Thr Tyr Ser Asp Val Cys Met Ala Pro Ala Ala
305                 310                 315                 320

Asp Met Phe Glu Glu Gly Val Lys Leu Gln Val Leu Lys Lys Gly Thr
                325                 330                 335

Met Phe Pro Ser Arg Ala Asn Lys Leu Tyr Glu Leu Phe Cys Lys Tyr
            340                 345                 350

Asp Ser Phe Glu Ser Met Pro Pro Ala Glu Leu Ala Arg Val Glu Lys
        355                 360                 365

Arg Ile Phe Ser Arg Ala Leu Glu Glu Val Trp Asp Glu Thr Lys Asn
    370                 375                 380

Phe Tyr Ile Asn Arg Leu His Asn Pro Glu Lys Ile Gln Arg Ala Glu
385                 390                 395                 400

Arg Asp Pro Lys Leu Lys Met Ser Leu Cys Phe Arg Trp Tyr Leu Ser
                405                 410                 415

Leu Ala Ser Arg Trp Ala Asn Thr Gly Ala Ser Asp Arg Val Met Asp
            420                 425                 430

Tyr Gln Val Trp Cys Gly Pro Ala Ile Gly Ser Phe Asn Asp Phe Ile
        435                 440                 445

Lys Gly Thr Tyr Leu Asp Pro Ala Val Ala Asn Glu Tyr Pro Cys Val
    450                 455                 460

Val Gln Ile Asn Lys Gln Ile Leu Arg Gly Ala Cys Phe Leu Arg Arg
465                 470                 475                 480

Leu Glu Ile Leu Arg Asn Ala Arg Leu Ser Asp Gly Ala Ala Ala Leu
                485                 490                 495

Val Ala Ser Ile Asp Asp Thr Tyr Val Pro Ala Glu Lys Leu
            500                 505                 510
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<210> SEQ ID NO 27
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1350)

<400> SEQUENCE: 27

atg gcc gct cgg aat gtg agc gcc gcg cat gag atg cac gat gaa aag      48
Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

cgc atc gcc gtc gtc ggc atg gcc gtc cag tac gcc gga tgc aaa acc      96
Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

aag gac gag ttc tgg gag gtg ctc atg aac ggc aag gtc gag tcc aag     144
Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

gtg atc agc gac aaa cga ctc ggc tcc aac tac cgc gcc gag cac tac     192
Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

aaa gca gag cgc agc aag tat gcc gac acc ttt tgc aac gaa acg tac     240
Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80

ggc acc ctt gac gag aac gag atc gac aac gag cac gaa ctc ctc ctc     288
Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95

aac ctc gcc aag cag gca ctc gca gag aca tcc gtc aaa gac tcg aca     336
Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

cgc tgc ggc atc gtc agc ggc tgc ctc tcg ttc ccc atg gac aac ctc     384
Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

cag ggt gaa ctc ctc aac gtg tac caa aac cat gtc gag aaa aag ctc     432
Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

ggg gcc cgc gtc ttc aag gac gcc tcc cat tgg tcc gaa cgc gag cag     480
Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

tcc aac aaa ccc gag gcc ggt gac cgc cgc atc ttc atg gac ccg gcc     528
Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

tcc ttc gtc gcc gaa gaa ctc aac ctc ggc gcc ctt cac tac tcc gtc     576
Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

gac gca gca tgc gcc acg gcg ctc tac gtg ctc cgc ctc gcg cag gat     624
Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205

cat ctc gtc tcc ggc gcc gcc gac gtc atg ctc tgc ggt gcc acc tgc     672
His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

ctg ccg gag ccc ttt ttc atc ctt tcg ggc ttt tcc acc ttc cag gcc     720
Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

atg ccc gtc ggc acg ggc cag aac gtg tcc atg ccg ctg cac aag gac     768
Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

agc cag ggc ctc acc ccg ggt gag ggc ggc tcc atc atg gtc ctc aag     816
Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270
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cgt ctc gat gat gcc atc cgc gac ggc gac cac att tac ggc acc ctt      864
Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

ctc ggc gcc aat gtc agc aac tcc ggc aca ggt ctg ccc ctc aag ccc      912
Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

ctt ctc ccc agc gag aaa aag tgc ctc atg gac acc tac acg cgc att      960
Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

aac gtg cac ccg cac aag att cag tac gtc gag tgc cac gcc acc ggc     1008
Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

acg ccc cag ggt gat cgt gtg gaa atc gac gcc gtc aag gcc tgc ttt     1056
Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

gaa ggc aag gtc ccc cgt ttc ggt acc aca aag ggc aac ttt gga cac     1104
Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

acc cts gyc gca gcc ggc ttt gcc ggt atg tgc aag gtc ctc ctc tcc     1152
Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380

atg aag cat ggc atc atc ccg ccc acc ccg ggt atc gat gac gag acc     1200
Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

aag atg gac cct ctc gtc gtc tcc ggt gag gcc atc cca tgg cca gag     1248
Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415

acc aac ggc gag ccc aag cgc gcc ggt ctc tcg gcc ttt ggc ttt ggt     1296
Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

ggc acc aac gcc cat gcc gtc ttt gag gag cat gac ccc tcc aac gcc     1344
Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

gcc tgc                                                             1350
Ala Cys
    450


<210> SEQ ID NO 28
<211> LENGTH: 450
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (370)..(370)
<223> OTHER INFORMATION: The 'Xaa' at location 370 stands for Leu.
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (371)..(371)
<223> OTHER INFORMATION: The 'Xaa' at location 371 stands for Ala, or
      Val.

<400> SEQUENCE: 28

Met Ala Ala Arg Asn Val Ser Ala Ala His Glu Met His Asp Glu Lys
1               5                   10                  15

Arg Ile Ala Val Val Gly Met Ala Val Gln Tyr Ala Gly Cys Lys Thr
            20                  25                  30

Lys Asp Glu Phe Trp Glu Val Leu Met Asn Gly Lys Val Glu Ser Lys
        35                  40                  45

Val Ile Ser Asp Lys Arg Leu Gly Ser Asn Tyr Arg Ala Glu His Tyr
    50                  55                  60

Lys Ala Glu Arg Ser Lys Tyr Ala Asp Thr Phe Cys Asn Glu Thr Tyr
65                  70                  75                  80
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Gly Thr Leu Asp Glu Asn Glu Ile Asp Asn Glu His Glu Leu Leu Leu
                85                  90                  95

Asn Leu Ala Lys Gln Ala Leu Ala Glu Thr Ser Val Lys Asp Ser Thr
            100                 105                 110

Arg Cys Gly Ile Val Ser Gly Cys Leu Ser Phe Pro Met Asp Asn Leu
        115                 120                 125

Gln Gly Glu Leu Leu Asn Val Tyr Gln Asn His Val Glu Lys Lys Leu
    130                 135                 140

Gly Ala Arg Val Phe Lys Asp Ala Ser His Trp Ser Glu Arg Glu Gln
145                 150                 155                 160

Ser Asn Lys Pro Glu Ala Gly Asp Arg Arg Ile Phe Met Asp Pro Ala
                165                 170                 175

Ser Phe Val Ala Glu Glu Leu Asn Leu Gly Ala Leu His Tyr Ser Val
            180                 185                 190

Asp Ala Ala Cys Ala Thr Ala Leu Tyr Val Leu Arg Leu Ala Gln Asp
        195                 200                 205

His Leu Val Ser Gly Ala Ala Asp Val Met Leu Cys Gly Ala Thr Cys
    210                 215                 220

Leu Pro Glu Pro Phe Phe Ile Leu Ser Gly Phe Ser Thr Phe Gln Ala
225                 230                 235                 240

Met Pro Val Gly Thr Gly Gln Asn Val Ser Met Pro Leu His Lys Asp
                245                 250                 255

Ser Gln Gly Leu Thr Pro Gly Glu Gly Gly Ser Ile Met Val Leu Lys
            260                 265                 270

Arg Leu Asp Asp Ala Ile Arg Asp Gly Asp His Ile Tyr Gly Thr Leu
        275                 280                 285

Leu Gly Ala Asn Val Ser Asn Ser Gly Thr Gly Leu Pro Leu Lys Pro
    290                 295                 300

Leu Leu Pro Ser Glu Lys Lys Cys Leu Met Asp Thr Tyr Thr Arg Ile
305                 310                 315                 320

Asn Val His Pro His Lys Ile Gln Tyr Val Glu Cys His Ala Thr Gly
                325                 330                 335

Thr Pro Gln Gly Asp Arg Val Glu Ile Asp Ala Val Lys Ala Cys Phe
            340                 345                 350

Glu Gly Lys Val Pro Arg Phe Gly Thr Thr Lys Gly Asn Phe Gly His
        355                 360                 365

Thr Xaa Xaa Ala Ala Gly Phe Ala Gly Met Cys Lys Val Leu Leu Ser
    370                 375                 380

Met Lys His Gly Ile Ile Pro Pro Thr Pro Gly Ile Asp Asp Glu Thr
385                 390                 395                 400

Lys Met Asp Pro Leu Val Val Ser Gly Glu Ala Ile Pro Trp Pro Glu
                405                 410                 415

Thr Asn Gly Glu Pro Lys Arg Ala Gly Leu Ser Ala Phe Gly Phe Gly
            420                 425                 430

Gly Thr Asn Ala His Ala Val Phe Glu Glu His Asp Pro Ser Asn Ala
        435                 440                 445

Ala Cys
    450
```

<210> SEQ ID NO 29
<211> LENGTH: 1500
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:

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```
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1500)

<400> SEQUENCE: 29

aag gtt cag ccc gtc ttt gcc aac ggc gcc gcc act gtc ggc ccc gag       48
Lys Val Gln Pro Val Phe Ala Asn Gly Ala Ala Thr Val Gly Pro Glu
1               5                   10                  15

gcc tcc aag gct tcc tcc ggc gcc agc gcc agc gcc agc gcc gcc ccg       96
Ala Ser Lys Ala Ser Ser Gly Ala Ser Ala Ser Ala Ser Ala Ala Pro
            20                  25                  30

gcc aag cct gcc ttc agc gcc gat gtt ctt gcg ccc aag ccc gtt gcc      144
Ala Lys Pro Ala Phe Ser Ala Asp Val Leu Ala Pro Lys Pro Val Ala
        35                  40                  45

ctt ccc gag cac atc ctc aag ggc gac gcc ctc gcc ccc aag gag atg      192
Leu Pro Glu His Ile Leu Lys Gly Asp Ala Leu Ala Pro Lys Glu Met
    50                  55                  60

tcc tgg cac ccc atg gcc cgc atc ccg ggc aac ccg acg ccc tct ttt      240
Ser Trp His Pro Met Ala Arg Ile Pro Gly Asn Pro Thr Pro Ser Phe
65                  70                  75                  80

gcg ccc tcg gcc tac aag ccg cgc aac atc gcc ttt acg ccc ttc ccc      288
Ala Pro Ser Ala Tyr Lys Pro Arg Asn Ile Ala Phe Thr Pro Phe Pro
                85                  90                  95

ggc aac ccc aac gat aac gac cac acc ccg ggc aag atg ccg ctc acc      336
Gly Asn Pro Asn Asp Asn Asp His Thr Pro Gly Lys Met Pro Leu Thr
            100                 105                 110

tgg ttc aac atg gcc gag ttc atg gcc ggc aag gtc agc atg tgc ctc      384
Trp Phe Asn Met Ala Glu Phe Met Ala Gly Lys Val Ser Met Cys Leu
        115                 120                 125

ggc ccc gag ttc gcc aag ttc gac gac tcg aac acc agc cgc agc ccc      432
Gly Pro Glu Phe Ala Lys Phe Asp Asp Ser Asn Thr Ser Arg Ser Pro
    130                 135                 140

gct tgg gac ctc gct ctc gtc acc cgc gcc gtg tct gtg tct gac ctc      480
Ala Trp Asp Leu Ala Leu Val Thr Arg Ala Val Ser Val Ser Asp Leu
145                 150                 155                 160

aag cac gtc aac tac cgc aac atc gac ctc gac ccc tcc aag ggt acc      528
Lys His Val Asn Tyr Arg Asn Ile Asp Leu Asp Pro Ser Lys Gly Thr
                165                 170                 175

atg gtc ggc gag ttc gac tgc ccc gcg gac gcc tgg ttc tac aag ggc      576
Met Val Gly Glu Phe Asp Cys Pro Ala Asp Ala Trp Phe Tyr Lys Gly
            180                 185                 190

gcc tgc aac gat gcc cac atg ccg tac tcg atc ctc atg gag atc gcc      624
Ala Cys Asn Asp Ala His Met Pro Tyr Ser Ile Leu Met Glu Ile Ala
        195                 200                 205

ctc cag acc tcg ggt gtg ctc acc tcg gtg ctc aag gcg ccc ctg acc      672
Leu Gln Thr Ser Gly Val Leu Thr Ser Val Leu Lys Ala Pro Leu Thr
    210                 215                 220

atg gag aag gac gac atc ctc ttc cgc aac ctc gac gcc aac gcc gag      720
Met Glu Lys Asp Asp Ile Leu Phe Arg Asn Leu Asp Ala Asn Ala Glu
225                 230                 235                 240

ttc gtg cgc gcc gac ctc gac tac cgc ggc aag act atc cgc aac gtc      768
Phe Val Arg Ala Asp Leu Asp Tyr Arg Gly Lys Thr Ile Arg Asn Val
                245                 250                 255

acc aag tgc act ggc tac agc atg ctc ggc gag atg ggc gtc cac cgc      816
Thr Lys Cys Thr Gly Tyr Ser Met Leu Gly Glu Met Gly Val His Arg
            260                 265                 270

ttc acc ttt gag ctc tac gtc gat gat gtg ctc ttt tac aag ggc tcg      864
Phe Thr Phe Glu Leu Tyr Val Asp Asp Val Leu Phe Tyr Lys Gly Ser
        275                 280                 285

acc tcg ttc ggc tgg ttc gtg ccc gag gtc ttt gcc gcc cag gcc ggc      912
Thr Ser Phe Gly Trp Phe Val Pro Glu Val Phe Ala Ala Gln Ala Gly
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    290                 295                 300

ctc gac aac ggc cgc aag tcg gag ccc tgg ttc att gag aac aag gtt      960
Leu Asp Asn Gly Arg Lys Ser Glu Pro Trp Phe Ile Glu Asn Lys Val
305                 310                 315                 320

ccg gcc tcg cag gtc tcc tcc ttt gac gtg cgc ccc aac ggc agc ggc     1008
Pro Ala Ser Gln Val Ser Ser Phe Asp Val Arg Pro Asn Gly Ser Gly
                325                 330                 335

cgc acc gcc atc ttc gcc aac gcc ccc agc ggc gcc cag ctc aac cgc     1056
Arg Thr Ala Ile Phe Ala Asn Ala Pro Ser Gly Ala Gln Leu Asn Arg
            340                 345                 350

cgc acg gac cag ggc cag tac ctc gac gcc gtc gac att gtc tcc ggc     1104
Arg Thr Asp Gln Gly Gln Tyr Leu Asp Ala Val Asp Ile Val Ser Gly
        355                 360                 365

agc ggc aag aag agc ctc ggc tac gcc cac ggt tcc aag acg gtc aac     1152
Ser Gly Lys Lys Ser Leu Gly Tyr Ala His Gly Ser Lys Thr Val Asn
    370                 375                 380

ccg aac gac tgg ttc ttc tcg tgc cac ttt tgg ttt gac tcg gtc atg     1200
Pro Asn Asp Trp Phe Phe Ser Cys His Phe Trp Phe Asp Ser Val Met
385                 390                 395                 400

ccc gga agt ctc ggt gtc gag tcc atg ttc cag ctc gtc gag gcc atc     1248
Pro Gly Ser Leu Gly Val Glu Ser Met Phe Gln Leu Val Glu Ala Ile
                405                 410                 415

gcc gcc cac gag gat ctc gct ggc aaa gca cgg cat tgc caa ccc cac     1296
Ala Ala His Glu Asp Leu Ala Gly Lys Ala Arg His Cys Gln Pro His
            420                 425                 430

ctt tgt gca cgc ccc cgg gca aga tca agc tgg aag tac cgc ggc cag     1344
Leu Cys Ala Arg Pro Arg Ala Arg Ser Ser Trp Lys Tyr Arg Gly Gln
        435                 440                 445

ctc acg ccc aag agc aag aag atg gac tcg gag gtc cac atc gtg tcc     1392
Leu Thr Pro Lys Ser Lys Lys Met Asp Ser Glu Val His Ile Val Ser
    450                 455                 460

gtg gac gcc cac gac ggc gtt gtc gac ctc gtc gcc gac ggc ttc ctc     1440
Val Asp Ala His Asp Gly Val Val Asp Leu Val Ala Asp Gly Phe Leu
465                 470                 475                 480

tgg gcc gac agc ctc cgc gtc tac tcg gtg agc aac att cgc gtg cgc     1488
Trp Ala Asp Ser Leu Arg Val Tyr Ser Val Ser Asn Ile Arg Val Arg
                485                 490                 495

atc gcc tcc ggt                                                     1500
Ile Ala Ser Gly
            500


<210> SEQ ID NO 30
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 30

Lys Val Gln Pro Val Phe Ala Asn Gly Ala Ala Thr Val Gly Pro Glu
1               5                   10                  15

Ala Ser Lys Ala Ser Ser Gly Ala Ser Ala Ser Ala Ser Ala Ala Pro
            20                  25                  30

Ala Lys Pro Ala Phe Ser Ala Asp Val Leu Ala Pro Lys Pro Val Ala
        35                  40                  45

Leu Pro Glu His Ile Leu Lys Gly Asp Ala Leu Ala Pro Lys Glu Met
    50                  55                  60

Ser Trp His Pro Met Ala Arg Ile Pro Gly Asn Pro Thr Pro Ser Phe
65                  70                  75                  80

Ala Pro Ser Ala Tyr Lys Pro Arg Asn Ile Ala Phe Thr Pro Phe Pro
                85                  90                  95
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Gly Asn Pro Asn Asp Asn Asp His Thr Pro Gly Lys Met Pro Leu Thr
            100                 105                 110

Trp Phe Asn Met Ala Glu Phe Met Ala Gly Lys Val Ser Met Cys Leu
        115                 120                 125

Gly Pro Glu Phe Ala Lys Phe Asp Asp Ser Asn Thr Ser Arg Ser Pro
    130                 135                 140

Ala Trp Asp Leu Ala Leu Val Thr Arg Ala Val Ser Val Ser Asp Leu
145                 150                 155                 160

Lys His Val Asn Tyr Arg Asn Ile Asp Leu Asp Pro Ser Lys Gly Thr
                165                 170                 175

Met Val Gly Glu Phe Asp Cys Pro Ala Asp Ala Trp Phe Tyr Lys Gly
            180                 185                 190

Ala Cys Asn Asp Ala His Met Pro Tyr Ser Ile Leu Met Glu Ile Ala
        195                 200                 205

Leu Gln Thr Ser Gly Val Leu Thr Ser Val Leu Lys Ala Pro Leu Thr
    210                 215                 220

Met Glu Lys Asp Asp Ile Leu Phe Arg Asn Leu Asp Ala Asn Ala Glu
225                 230                 235                 240

Phe Val Arg Ala Asp Leu Asp Tyr Arg Gly Lys Thr Ile Arg Asn Val
                245                 250                 255

Thr Lys Cys Thr Gly Tyr Ser Met Leu Gly Glu Met Gly Val His Arg
            260                 265                 270

Phe Thr Phe Glu Leu Tyr Val Asp Asp Val Leu Phe Tyr Lys Gly Ser
        275                 280                 285

Thr Ser Phe Gly Trp Phe Val Pro Glu Val Phe Ala Ala Gln Ala Gly
    290                 295                 300

Leu Asp Asn Gly Arg Lys Ser Glu Pro Trp Phe Ile Glu Asn Lys Val
305                 310                 315                 320

Pro Ala Ser Gln Val Ser Ser Phe Asp Val Arg Pro Asn Gly Ser Gly
                325                 330                 335

Arg Thr Ala Ile Phe Ala Asn Ala Pro Ser Gly Ala Gln Leu Asn Arg
            340                 345                 350

Arg Thr Asp Gln Gly Gln Tyr Leu Asp Ala Val Asp Ile Val Ser Gly
        355                 360                 365

Ser Gly Lys Lys Ser Leu Gly Tyr Ala His Gly Ser Lys Thr Val Asn
    370                 375                 380

Pro Asn Asp Trp Phe Phe Ser Cys His Phe Trp Phe Asp Ser Val Met
385                 390                 395                 400

Pro Gly Ser Leu Gly Val Glu Ser Met Phe Gln Leu Val Glu Ala Ile
                405                 410                 415

Ala Ala His Glu Asp Leu Ala Gly Lys Ala Arg His Cys Gln Pro His
            420                 425                 430

Leu Cys Ala Arg Pro Arg Ala Arg Ser Ser Trp Lys Tyr Arg Gly Gln
        435                 440                 445

Leu Thr Pro Lys Ser Lys Lys Met Asp Ser Glu Val His Ile Val Ser
    450                 455                 460

Val Asp Ala His Asp Gly Val Val Asp Leu Val Ala Asp Gly Phe Leu
465                 470                 475                 480

Trp Ala Asp Ser Leu Arg Val Tyr Ser Val Ser Asn Ile Arg Val Arg
                485                 490                 495

Ile Ala Ser Gly
            500
```

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<210> SEQ ID NO 31
<211> LENGTH: 1512
<212> TYPE: DNA
<213> ORGANISM: Schizochytrium sp.
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1512)

<400> SEQUENCE: 31

gcc ccg ctc tac ctc tcg cag gac ccg acc agc ggc cag ctc aag aag       48
Ala Pro Leu Tyr Leu Ser Gln Asp Pro Thr Ser Gly Gln Leu Lys Lys
1               5                   10                  15

cac acc gac gtg gcc tcc ggc cag gcc acc atc gtg cag ccc tgc acg       96
His Thr Asp Val Ala Ser Gly Gln Ala Thr Ile Val Gln Pro Cys Thr
            20                  25                  30

ctc ggc gac ctc ggt gac cgc tcc ttc atg gag acc tac ggc gtc gtc      144
Leu Gly Asp Leu Gly Asp Arg Ser Phe Met Glu Thr Tyr Gly Val Val
        35                  40                  45

gcc ccg ctg tac acg ggc gcc atg gcc aag ggc att gcc tcg gcg gac      192
Ala Pro Leu Tyr Thr Gly Ala Met Ala Lys Gly Ile Ala Ser Ala Asp
    50                  55                  60

ctc gtc atc gcc gcc ggc aag cgc aag atc ctc ggc tcc ttt ggc gcc      240
Leu Val Ile Ala Ala Gly Lys Arg Lys Ile Leu Gly Ser Phe Gly Ala
65                  70                  75                  80

ggc ggc ctc ccc atg cac cac gtg cgc gcc gcc ctc gag aag atc cag      288
Gly Gly Leu Pro Met His His Val Arg Ala Ala Leu Glu Lys Ile Gln
                85                  90                  95

gcc gcc ctg cct cag ggc ccc tac gcc gtc aac ctc atc cac tcg cct      336
Ala Ala Leu Pro Gln Gly Pro Tyr Ala Val Asn Leu Ile His Ser Pro
            100                 105                 110

ttt gac agc aac ctc gag aag ggc aac gtc gat ctc ttc ctc gag aag      384
Phe Asp Ser Asn Leu Glu Lys Gly Asn Val Asp Leu Phe Leu Glu Lys
        115                 120                 125

ggc gtc act gtg gtg gag gcc tcg gca ttc atg acc ctc acc ccg cag      432
Gly Val Thr Val Val Glu Ala Ser Ala Phe Met Thr Leu Thr Pro Gln
    130                 135                 140

gtc gtg cgc tac cgc gcc gcc ggc ctc tcg cgc aac gcc gac ggt tcg      480
Val Val Arg Tyr Arg Ala Ala Gly Leu Ser Arg Asn Ala Asp Gly Ser
145                 150                 155                 160

gtc aac atc cgc aac cgc atc atc ggc aag gtc tcg cgc acc gag ctc      528
Val Asn Ile Arg Asn Arg Ile Ile Gly Lys Val Ser Arg Thr Glu Leu
                165                 170                 175

gcc gag atg ttc atc cgc ccg gcc ccg gag cac ctc ctc gag aag ctc      576
Ala Glu Met Phe Ile Arg Pro Ala Pro Glu His Leu Leu Glu Lys Leu
            180                 185                 190

atc gcc tcg ggc gag atc acc cag gag cag gcc gag ctc gcg cgc cgc      624
Ile Ala Ser Gly Glu Ile Thr Gln Glu Gln Ala Glu Leu Ala Arg Arg
        195                 200                 205

gtt ccc gtc gcc gac gat atc gct gtc gag gct gac tcg ggc ggc cac      672
Val Pro Val Ala Asp Asp Ile Ala Val Glu Ala Asp Ser Gly Gly His
    210                 215                 220

acc gac aac cgc ccc atc cac gtc atc ctc ccg ctc atc atc aac ctc      720
Thr Asp Asn Arg Pro Ile His Val Ile Leu Pro Leu Ile Ile Asn Leu
225                 230                 235                 240

cgc aac cgc ctg cac cgc gag tgc ggc tac ccc gcg cac ctc cgc gtc      768
Arg Asn Arg Leu His Arg Glu Cys Gly Tyr Pro Ala His Leu Arg Val
                245                 250                 255

cgc gtt ggc gcc ggc ggt ggc gtc ggc tgc ccg cag gcc gcc gcc gcc      816
Arg Val Gly Ala Gly Gly Gly Val Gly Cys Pro Gln Ala Ala Ala Ala
            260                 265                 270
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gcg ctc acc atg ggc gcc gcc ttc atc gtc acc ggc act gtc aac cag      864
Ala Leu Thr Met Gly Ala Ala Phe Ile Val Thr Gly Thr Val Asn Gln
        275                 280                 285

gtc gcc aag cag tcc ggc acc tgc gac aac gtg cgc aag cag ctc tcg      912
Val Ala Lys Gln Ser Gly Thr Cys Asp Asn Val Arg Lys Gln Leu Ser
    290                 295                 300

cag gcc acc tac tcg gat atc tgc atg gcc ccg gcc gcc gac atg ttc      960
Gln Ala Thr Tyr Ser Asp Ile Cys Met Ala Pro Ala Ala Asp Met Phe
305                 310                 315                 320

gag gag ggc gtc aag ctc cag gtc ctc aag aag gga acc atg ttc ccc     1008
Glu Glu Gly Val Lys Leu Gln Val Leu Lys Lys Gly Thr Met Phe Pro
                325                 330                 335

tcg cgc gcc aac aag ctc tac gag ctc ttt tgc aag tac gac tcc ttc     1056
Ser Arg Ala Asn Lys Leu Tyr Glu Leu Phe Cys Lys Tyr Asp Ser Phe
            340                 345                 350

gac tcc atg cct cct gcc gag ctc gag cgc atc gag aag cgt atc ttc     1104
Asp Ser Met Pro Pro Ala Glu Leu Glu Arg Ile Glu Lys Arg Ile Phe
        355                 360                 365

aag cgc gca ctc cag gag gtc tgg gag gag acc aag gac ttt tac att     1152
Lys Arg Ala Leu Gln Glu Val Trp Glu Glu Thr Lys Asp Phe Tyr Ile
    370                 375                 380

aac ggt ctc aag aac ccg gag aag atc cag cgc gcc gag cac gac ccc     1200
Asn Gly Leu Lys Asn Pro Glu Lys Ile Gln Arg Ala Glu His Asp Pro
385                 390                 395                 400

aag ctc aag atg tcg ctc tgc ttc cgc tgg tac ctt ggt ctt gcc agc     1248
Lys Leu Lys Met Ser Leu Cys Phe Arg Trp Tyr Leu Gly Leu Ala Ser
                405                 410                 415

cgc tgg gcc aac atg ggc gcc ccg gac cgc gtc atg gac tac cag gtc     1296
Arg Trp Ala Asn Met Gly Ala Pro Asp Arg Val Met Asp Tyr Gln Val
            420                 425                 430

tgg tgt ggc ccg gcc att ggc gcc ttc aac gac ttc atc aag ggc acc     1344
Trp Cys Gly Pro Ala Ile Gly Ala Phe Asn Asp Phe Ile Lys Gly Thr
        435                 440                 445

tac ctc gac ccc gct gtc tcc aac gag tac ccc tgt gtc gtc cag atc     1392
Tyr Leu Asp Pro Ala Val Ser Asn Glu Tyr Pro Cys Val Val Gln Ile
    450                 455                 460

aac ctg caa atc ctc cgt ggt gcc tgc tac ctg cgc cgt ctc aac gcc     1440
Asn Leu Gln Ile Leu Arg Gly Ala Cys Tyr Leu Arg Arg Leu Asn Ala
465                 470                 475                 480

ctg cgc aac gac ccg cgc att gac ctc gag acc gag gat gct gcc ttt     1488
Leu Arg Asn Asp Pro Arg Ile Asp Leu Glu Thr Glu Asp Ala Ala Phe
                485                 490                 495

gtc tac gag ccc acc aac gcg ctc                                     1512
Val Tyr Glu Pro Thr Asn Ala Leu
            500


<210> SEQ ID NO 32
<211> LENGTH: 504
<212> TYPE: PRT
<213> ORGANISM: Schizochytrium sp.

<400> SEQUENCE: 32

Ala Pro Leu Tyr Leu Ser Gln Asp Pro Thr Ser Gly Gln Leu Lys Lys
1               5                   10                  15

His Thr Asp Val Ala Ser Gly Gln Ala Thr Ile Val Gln Pro Cys Thr
            20                  25                  30

Leu Gly Asp Leu Gly Asp Arg Ser Phe Met Glu Thr Tyr Gly Val Val
        35                  40                  45

Ala Pro Leu Tyr Thr Gly Ala Met Ala Lys Gly Ile Ala Ser Ala Asp
    50                  55                  60
```

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Leu Val Ile Ala Ala Gly Lys Arg Lys Ile Leu Gly Ser Phe Gly Ala
65                  70                  75                  80

Gly Gly Leu Pro Met His His Val Arg Ala Ala Leu Glu Lys Ile Gln
                85                  90                  95

Ala Ala Leu Pro Gln Gly Pro Tyr Ala Val Asn Leu Ile His Ser Pro
            100                 105                 110

Phe Asp Ser Asn Leu Glu Lys Gly Asn Val Asp Leu Phe Leu Glu Lys
        115                 120                 125

Gly Val Thr Val Val Glu Ala Ser Ala Phe Met Thr Leu Thr Pro Gln
    130                 135                 140

Val Val Arg Tyr Arg Ala Ala Gly Leu Ser Arg Asn Ala Asp Gly Ser
145                 150                 155                 160

Val Asn Ile Arg Asn Arg Ile Ile Gly Lys Val Ser Arg Thr Glu Leu
                165                 170                 175

Ala Glu Met Phe Ile Arg Pro Ala Pro Glu His Leu Leu Glu Lys Leu
            180                 185                 190

Ile Ala Ser Gly Glu Ile Thr Gln Glu Gln Ala Glu Leu Ala Arg Arg
        195                 200                 205

Val Pro Val Ala Asp Asp Ile Ala Val Glu Ala Asp Ser Gly Gly His
    210                 215                 220

Thr Asp Asn Arg Pro Ile His Val Ile Leu Pro Leu Ile Ile Asn Leu
225                 230                 235                 240

Arg Asn Arg Leu His Arg Glu Cys Gly Tyr Pro Ala His Leu Arg Val
                245                 250                 255

Arg Val Gly Ala Gly Gly Gly Val Gly Cys Pro Gln Ala Ala Ala Ala
            260                 265                 270

Ala Leu Thr Met Gly Ala Ala Phe Ile Val Thr Gly Thr Val Asn Gln
        275                 280                 285

Val Ala Lys Gln Ser Gly Thr Cys Asp Asn Val Arg Lys Gln Leu Ser
    290                 295                 300

Gln Ala Thr Tyr Ser Asp Ile Cys Met Ala Pro Ala Ala Asp Met Phe
305                 310                 315                 320

Glu Glu Gly Val Lys Leu Gln Val Leu Lys Lys Gly Thr Met Phe Pro
                325                 330                 335

Ser Arg Ala Asn Lys Leu Tyr Glu Leu Phe Cys Lys Tyr Asp Ser Phe
            340                 345                 350

Asp Ser Met Pro Pro Ala Glu Leu Glu Arg Ile Glu Lys Arg Ile Phe
        355                 360                 365

Lys Arg Ala Leu Gln Glu Val Trp Glu Glu Thr Lys Asp Phe Tyr Ile
    370                 375                 380

Asn Gly Leu Lys Asn Pro Glu Lys Ile Gln Arg Ala Glu His Asp Pro
385                 390                 395                 400

Lys Leu Lys Met Ser Leu Cys Phe Arg Trp Tyr Leu Gly Leu Ala Ser
                405                 410                 415

Arg Trp Ala Asn Met Gly Ala Pro Asp Arg Val Met Asp Tyr Gln Val
            420                 425                 430

Trp Cys Gly Pro Ala Ile Gly Ala Phe Asn Asp Phe Ile Lys Gly Thr
        435                 440                 445

Tyr Leu Asp Pro Ala Val Ser Asn Glu Tyr Pro Cys Val Val Gln Ile
    450                 455                 460

Asn Leu Gln Ile Leu Arg Gly Ala Cys Tyr Leu Arg Arg Leu Asn Ala
465                 470                 475                 480
```

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Leu Arg Asn Asp Pro Arg Ile Asp Leu Glu Thr Glu Asp Ala Ala Phe
                485                 490                 495

Val Tyr Glu Pro Thr Asn Ala Leu
            500


<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: motif
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(3)
<223> OTHER INFORMATION: x = any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: x = A or S
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: x = any amino acid

<400> SEQUENCE: 33

Trp Xaa Xaa Lys Glu Xaa Xaa Xaa Lys
1               5


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<223> OTHER INFORMATION: n = a, c, g, or t

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What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:6 or that is an enzymatically active fragment of SEQ ID NO:6, wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

2. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is an enzymatically active fragment of SEQ ID NO:6, wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

3. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:6, wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

4. The isolated nucleic acid molecule of claim 1, consisting of a nucleic acid sequence encoding an amino acid sequence that is at least 95% identical to SEQ ID NO:6, wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

5. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is at least 96% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

6. The isolated nucleic acid molecule of claim 1, consisting of a nucleic acid sequence encoding an amino acid sequence that is at least 96% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

7. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is at least 97% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

8. The isolated nucleic acid molecule of claim 1, consisting of a nucleic acid sequence encoding an amino acid sequence that is at least 97% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

9. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is at least 98% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

10. The isolated nucleic acid molecule of claim 1, consisting of a nucleic acid sequence encoding an amino acid sequence that is at least 98% identical to SEQ ID NO:6 wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

11. The isolated nucleic acid molecule of claim 1, comprising a nucleic acid sequence encoding an amino acid sequence that is at least 99% identical to SEQ ID NO:6, wherein said

amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

12. The isolated nucleic acid molecule of claim **1**, consisting of a nucleic acid sequence encoding an amino acid sequence that is at least 99% identical to SEQ ID NO:6, wherein said amino acid sequence has FabA-like β-hydroxy acyl-ACP dehydrase activity and enoyl ACP-reductase activity.

13. The isolated nucleic acid molecule of claim **1**, comprising a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:6.

14. The isolated nucleic acid molecule of claim **1**, consisting of a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:6.

15. The isolated nucleic acid molecule of claim **1**, wherein the nucleic acid molecule comprises SEQ ID NO:5.

16. The isolated nucleic acid molecule of claim **1**, wherein the nucleic acid molecule consists of SEQ ID NO:5.

17. A recombinant nucleic acid molecule comprising the nucleic acid molecule of claim **1** and a transcription control sequence.

18. A recombinant plant cell that expresses the nucleic acid molecule of claim **1**.

19. A recombinant microbial cell that expresses a recombinant vector comprising the nucleic acid molecule of claim **1** and a transcription control sequence.

20. The recombinant microbial cell of claim **19**, wherein the microbial cell is a bacterium.

21. The recombinant microbial cell of claim **19**, wherein the microbial cell is a Thraustochytriales microorganism.

22. The recombinant microbial cell of claim **21**, wherein the Thraustochytriales microorganism is a *Schizochytrium* or a *Thraustochytrium.*

23. A method to produce at least one polyunsaturated fatty acid (PUFA), comprising culturing under conditions effective to produce the PUFA, a plant cell or a microbial cell that expresses a PKS system for production of PUFAs, wherein the plant cell or microbial cell expresses a recombinant vector comprising the nucleic acid molecule of claim **1**.

* * * * *